A Contribution to the Embryology, Life-history, and Classification of the Dicyemids.

By

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With Plates 1—5.

Since the discovery of the strange parasites of the renal organ of Cephalopods, by Krohn, in 1839[1], they have been studied by Erdl, Kölliker, G. Wagener, Claparède, J. Müller, Ray Lankester, P. J. Van Beneden, and Ed. Van Beneden and his pupils, Foettinger and Moreau. It was Kölliker who first made known the highly interesting fact that these animals produce two kinds of embryos, and who, for this reason, gave them the name *Dicyema*[2]; but it is Ed. Van Beneden to whom we are chiefly indebted for what has thus far been ascertained concerning their embryology, classification, and systematic affinities.

It is quite unnecessary to give here the usual historical survey of results reached by previous investigators, as this has already been done by Van Beneden: and it would certainly be superfluous to offer a summary of the researches on this subject by Van Beneden himself, since every naturalist is familiar with his memoir on the Dicyemids, and most of our zoological text-books contain an epitome of the facts and conclusions there presented; and all the more so, since I shall have frequent occasion to refer to the same, by direct citations, in the body of this paper.

The important systematic position assigned to this group of parasites by Van Beneden, and the many questions still unanswered in re-

[1] Not 1830 as has been so often stated.

[2] dis and ζυγμα, embryo.

gard to their cycle of life, seemed to me to render further investigation desirable, even if nothing more than a confirmation of results already reached should be accomplished. Although the question of cardinal interest — the fate of the »Infusoriform embryo« — still remains unsolved, yet some facts of considerable importance have been obtained.

My study of the Dicyemids, begun in December, 1881, and continued until the end of April of the current year, has been carried on in the Zoological Station at Naples. For the opportunity and the rare facilities for work which I have enjoyed in this institution, I am more deeply indebted than any of the conventional forms of acknowledgment would indicate. To pass over my obligations in silence might be construed as a failure to recognize them; while any attempt to express them would certainly seem inadequate to myself and extravagant to those unacquainted with the circumstances. I hope the acknowledgment of the difficulty will be accepted as the best evidence I can, at present, give of my appreciation of the advantages enjoyed while at the Station, and of my gratitude for the cordial and generous treatment received from its Director and all the gentlemen associated in its management.

As to the direction my study has taken, I may say that, at the outset, I did not anticipate being drawn into systematic work; and, had I foreseen the necessity for such work and the amount of time and labor involved in it, it is more than probable that I should have devoted my attention to another subject. Still this portion of my work has not been all drudgery. The accurate determination of species calls for a careful discrimination between individual peculiarities and specific characters; and the range of variability, in both these respects, is a question not devoid of interest.

The methods employed have been the same as those described by Van Beneden. I have used acetic acid quite as much as osmic acid, but have found it important to watch the action of these re-agents on fresh specimens. I have found the picro-carmine solution described by Mayer to be a very useful staining fluid.

The matter to be dealt with in this paper may be arranged under four heads:

1. Classification, including historical and critical remarks, and systematic descriptions.

2. Reproduction, embracing the phenomena of transition from the rhombogenic to the nematogenic condition, a comparison of the Dicyemids with the Orthonectidae, and a general survey of the evolutionary cycle, so far as at present known.

3. The development of the vermiform embryo, and the origin of the germ-cells, with remarks on endogenous cell-formation.

4. Systematic affinities of the Dicyemids.

Chapter I.

The Classification of the Dicyemidae.

Historical.

Kölliker supposed that all Dicyemids could be included in one species, for which he proposed the name *Dicyema paradoxum*.

Wagener introduced two specific names, one (*D. Eledones*) for the Dicyemids found in *Eledone moschata*, the other (*D. gracile*) for those living in *Sepia officinalis*. Wagener remarks that the Dicyemids of *Octopus* and *Sepiola* resemble closely those of *Eledone*; but he does not affirm that they are to be included under the same specific name, as represented by Van Beneden.

Appended to Wagener's paper, comes a description by Claparède of a species found by himself, Lachmann, and J. Müller, in *Eledone cirrosa*, from the coasts of Norway, under the name *Dicyema Mülleri*. No further progress was made in the classification of these animals until the investigations of Van Beneden were begun in 1874. The result of his observations in this direction may be seen from the table given below.

Dicyema Köll.	*D. typus* Ed. V. Ben.	in *Octopus vulgaris*.
	D. Clausiana Ed. V. Ben.	- - *macropus*.
Dicyemella Ed. V. Ben.	*D. Wageneri* Ed. V. Ben.	- *Eledone moschata*
	D. Mülleri Clap.	- - *cirrosa*.
Dicyemina Ed. V. Ben.	*D. Köllikeriana* Ed. V. Ben.	- *Sepia officinalis*.
	D. Schulziana Ed. V. Ben.	- - *biserialis*.
Dicyemopsis Ed. V. Ben.	*D. macrocephalus* Ed. V. Ben.	- *Sepiola Rondeletii*.

Van Beneden divides the Dicyemidae into four genera, and enumerates seven species, six of which appear under his own name. I have increased the number of species to ten, and included all in two genera, as will be seen from the following table.

Dicyema Köll.	1. *D. typus* Ed. V. Ben.	in *Octopus vulgaris* Lamark.
	2. *D. Clausianum* Ed. V. Ben.	- - *macropus* Risso.
	3. *D. microcephalum* Whit.	- - *De-Filippi* Verany.
	4. *D. moschatum* Whit.	- *Eledone moschata* Leach.
	5. *D. truncatum* Whit.	- *Sepia officinalis* Linn. - *elegans* Blain. *Rossia macrosoma* D.Chiaie.
	6. *D. Schulzianum* E. V. Ben.	- *Sepia biserialis* D. de Mont.
	7. *D. macrocephalum* E V.Ben.	- *Sepiola Rondeletii* Gesner.

Dicyemennea Whit. { 8. *D. Eledones* Wag. in { *Eledone Aldrovandi* D. Ch. / *Eledone moschata* Leach.
9. *D. Mülleri* Clap. - - *cirrosa* Lamark.
10. *D. gracile* Wag. - *Sepia officinalis* Linn.

Of the ten species here tabulated, Ed. Van Beneden has determined four, and Claparède one: of the five remaining, one, found in *O. De-Filippi*, is entirely new: two, occurring in *E. moschata*, have hitherto been regarded as one species, and named *Dicyema Eledones* by Wagener, *Dicyemella Wageneri* by Van Beneden; and the other two, generally found together in *Sepia officinalis*, have likewise been confounded by Wagener under the name *Dicyema gracile*, by Van Beneden under the name *Dicyemina Köllikeriana*.

With reference to naming the four species hitherto described as two, it was necessary, first of all, to decide what use could be made of the names already invented. After carefully considering all the statements bearing on the question, I have no hesitation in declaring the two names originating with Van Beneden synonymous with those introduced by Wagener; and, as in systematic zoölogy the claims of priority are not to be superseded by those founded on accuracy of description, it seems necessary to discard this part of Van Beneden's terminology. It is scarcely necessary to add, that I fully appreciate the superior merit of Van Beneden's descriptions as compared with those of Wagener; but usage does not allow such a discrimination to outweigh other considerations. Van Beneden gives no reasons for rejecting the names of his distinguished predecessor, and I fail to see on what grounds such liberty could have been justly taken. Possibly the erroneous opinion, which Van Beneden appears to have entertained, that Wagener intended the name, *D. Eledones*, not only for the Dicyemidae of *E. moschata* but also those of *Octopus* and *Sepiola*, may, in a measure, explain what, under any other supposition, must have been known to be a plain violation of the universally recognized law of priority. But this explanation could not apply to Wagener's second name, *D. gracile*, and thus there seems no escape from the conclusion that these names were discarded quite arbitrarily. It is hardly to be supposed that the imperfection of Wagener's diagnoses, could have had any decisive influence in this matter, so long as they furnished the means of certain identification. Van Beneden's studies led him to the conclusion that each Cephalopod has a single species of Dicyema, and this conviction seemed to him so well founded, that he made it the corner-stone of his whole scheme of classification, as I shall presently show. Accord-

ing to this view, the species of Dicyema would be determined the moment that of the Cephalopod became known, and vice versa. It is evident therefore that WAGENER gave, in naming the Cephalopod, all that was actually required by VAN BENEDEN's system for identification. I find no difficulty in recognizing in two of WAGENER's figures the two species of Dicyema which I have found in *E. moschata*; and precisely the same may be said of two figures given by VAN BENEDEN (Pl. 1, fig. 4 and Pl. 2, fig. 1).

The grounds for not adopting the names *D. Wageneri* and *D. Köllikeriana* are sufficiently obvious, without urging the additional fact that VAN BENEDEN has, in both cases, confounded two species under the same name.

With regard to the prior designations of WAGENER, the discovery that each covers two species, gives me a discretional right of rejecting or retaining them. Although I can no longer employ them in their original sense, still it seems possible to make use of them without causing any appreciable amount of confusion in nomenclature; and this course commends itself as one which accords best with the deference due to an eminent authority, and as one which smacks least of the practices of the reckless system-smasher and name-monger.

The course adopted with reference to the three generic names introduced by VAN BENEDEN, requires a brief explanation. It was impossible to retain these names without assenting to the conclusions which formed the basis of VAN BENEDEN's system of classification and determined his method of naming; for the names, method, and ideas cleave to each other with such logical coherence that they must stand or fall together. As these conclusions were not confirmed, but plainly contradicted by facts to be recorded in this paper, they had to be abandoned, and with them the generic names founded on them. It seems therefore proper to state precisely what these conclusions are, and how they stand related to the names under consideration.

With reference to the first point, the following citation is made:

»Mes études sur ces organismes me mettent en mesure d'affirmer que chaque Céphalopode a son espèce particulière de *Dicyema*. Mais les espèces qui habitent des Céphalopodes proches parents sont beaucoup plus voisines que celles qu'hébergent des Céphalopodes appartenant à des familles différentes. De là la nécessité d'établir plusieurs coupes génériques.« (1, p. 8.)

In harmony herewith, a system of names is invented which runs parallel with that of the Cephalopoda. The genus of the parasite coin-

cides with that of its host: and thus its limits are fixed, not by any morphological boundary lines discoverable in the parasites themselves, but indirectly through their assumed coincidence with the limits assigned to a totally foreign genus.

To the genus Octopus corresponds the genus *Dicyema*; to *Eledone*, *Dicyemella*; to *Sepia*, *Dicyemina*; and to *Sepiola*, *Dicyemopsis*. Such is the facile method adopted by Van Beneden in carving out a new system of generic names. Four genera were thus set up, and not a single generic character pointed out! If the correspondence affirmed really existed, it would be a very remarkable fact, and, I believe, quite an exceptional one: at all events, it would be preposterous to suppose that it could be accepted as a fact without some substantial evidence. I have to confess therefore to some surprise that van Beneden has neglected to offer any proof whatever in support of the position he has taken on this question, especially as on the assumed parallelism hangs the validity of his whole system of classification. The seven species given in his paper are described with more or less detail; but I find no allusion to g e n e r i c distinctions beyond what can be gathered from the passage before cited. Presumably the descriptions of the species include both specific and generic characters, but the line is nowhere distinctly drawn between the two.

Two distinct assertions are made in the above quotation:

1. Each Cephalopod has a single species of Dicyema.

2. The species found in closely allied Cephalopoda are much more nearly related than those found in Cephalopoda belonging to different families.

My observations warrant me in saying that neither of these assertions is correct.

In the description of species which is to follow the following facts will be established:

1. Two Cephalopods, *E. moschata* and *S. officinalis*, have each two species of Dicyema.

2. One species of Dicyema occurs in at least two different species of Cephalopod, while another is found in at least three different Cephalopods.

3. Two species, *D. Eledones* Wag. and *D. gracile* Wag., found in Cephalopods belonging to two different families (*E. moschata* and *S. officinalis*) differ from each other less than they differ from the species with which they are associated.

4. In Rossia and Sepiola, two genera of the Myopsidae standing,

according to Brock (24) in the closest genetic relationship, are found two species of Dicyema differing from each other as widely as either differs from *D. moschatum* of *E. moschata*.

These facts make it perfectly clear that I could not pursue the method of grouping adopted by Van Beneden, nor accept the generic divisions to which his erroneous views had given rise.

All the Dicyemids at present known may be easily and conveniently divided into two groups or genera, distinguishable by the number of cells in the head — polar cells of van Beneden. The first genus, to which the name *Dicyema*, originating with Kölliker, will be applied, includes seven species, all with eight polar cells. The second genus embraces three species, all characterized by nine polar cells, and may therefore be designated, *Dicyemennea*[1].

Explanatory Remarks.

Passing over histological details, which appear to have been very accurately given by Van Beneden, I shall here point out those characters by which the genera and species at present known may be most readily recognized; and, in so doing, shall adhere to the terminology employed by Van Beneden, so far as this meets my purpose. To avoid repetition and periphrases, a few explanatory remarks and definitions of the more frequently recurring terms are here introduced.

For systematic purposes, the cephalic enlargement is by far the most important part of the animal, as it furnishes the greater number of the generic and specific characters. The eight, or nine cells composing this portion, are always in two sets, and the first set invariably contains four cells. These two sets of polar cells form together the polar calotte (»coiffe polaire«, V. Ben.). The cells of the two sets may be designated according to their relative position, which is invariable in young individuals and persistent in the majority of species, pro-polar and meta-polar. In some cases, the pro-polar cells become, in the adults, central, and the meta-polar, peripheral. The bilateral symmetry of the calotte, first recognized by Van Beneden, will necessitate further the use of the terms dorsal, ventral, and lateral. The extremity of the body adjoining the calotte, is universally formed of two

[1] Dicyema and ἐννέα, nine. I should add that there is some doubt about the position of *D. Mülleri*, as Claparède failed to determine the number of polar cells.

lateral cells, called para-polar cells by Van Beneden. The caudal extremity is also formed of two cells, which, in young individuals at least, generally have a dorso-ventral position — a position at right angles to that of the parapolar cells.

The calotte, and the parapolar cells together with all the other external cells of the body, form the ectoderm, which is everywhere in immediate contact with an internal axial cell, as Van Beneden has already clearly shown. This axial cell may be said to represent the endoderm, in view of its position and embryonic history. In origin, structure, and general appearance, it is the same in each species, and need not therefore occupy our attention in the following descriptions. The ectoderm furnishes all the diagnostic characters: the number of cells composing the calotte determines the genus; while the size of these cells, both relative and absolute, their shape, relative position, axial relations, the whole number of ectodermal cells, the length of adult individuals, the form of the parapolar and caudal cells, etc., will supply the means of distinguishing species.

The genus *Dicyema* Köll., as already remarked, includes those species in which the calotte consists of eight cells, in two sets of four each. The octamerous calotte shows in most species a more or less decided tendency to obliquity, which as a rule increases with age, and often becomes so pronounced in the adults of some species, that all the eight cells take part in forming the ventral face. This is evidently an acquired character, since up to the time of birth, and often much later, it is entirely absent. Van Beneden goes altogether too far when he states (p. 17) that this obliquity is entirely due to a difference in size between the dorsal and ventral cells. The explanation of the origin of this peculiarity appears to me to lie in quite another direction. In the youngest individuals, the calotte exhibits a symmetry so complete that it is difficult to distinguish between the dorsal and the ventral side. At this stage there are obviously two planes coinciding with the axis of the body — a vertical and a horizontal one —, which divide the head into two like parts. The junction of the calotte with the body forms a vertical plane cutting the main axis at right angles. This form of the polar calotte, in which the line formed by the intersection of the two dividing planes is a direct prolongation of the axis of the body, may be called orthotropal. The orthotropal calotte passes into a plagiotropal form, by an unlike growth, not between the dorsal and ventral polar cells, but between the dorsal and ventral sides of the body. The dorsal side elongates more rapidly than the ventral, and the dorsal polar cells are thus

pushed forward until, in extreme cases, all the polar cells come to lie in the same oblique plane, which looks toward the ventral side.

The genus *Dicyemennea* embraces those species in which the calotte is composed of nine cells, four propolar and five metapolar. *D. Mülleri*, one of the three species included in this genus, is somewhat doubtfully placed, as the number of cells in the calotte has not been ascertained. The form of the calotte, as given in the figures of Claparède and Lachmann, makes it quite certain that it does not belong to the octamerous type; while its close resemblance to the enneamerous calotte of *D. Eledones* leaves but little doubt in regard to its generic position.

The difference of a single polar cell might, at first, appear insufficient for a generic mark, especially when estimated by any standard that would apply to one of the higher animals. Any objection of this kind will probably disappear on reflecting that every cell in a pluricellular animal has, in addition to its morphological and physiological value, a numerical and topical value. The numerical value stands in inverse ratio to the total number of cells composing the organism, and where the latter never exceeds 25—30 cells, the former must be very considerable. The topical value will also be greater in proportion as the total number of cells is smaller and the differentiated regions fewer; and, further, greater in proportion to the morphological and physiological importance of the region in which it is placed. The calotte of a Dicyema is the most highly differentiated part of the animal, and hence a difference of a single cell in its composition must be admitted to be of more importance to the systematist than the same numerical difference in any other region. A comparison of the octamerous with the enneamerous calotte will show that we have not over-estimated the fundamental difference between them. The added cell gives a calotte distinctly characterized in form and structure, and further distinguished by being permanently orthotropal.

Systematic Descriptions.

1. Dicyema typus E. v. Ben. Figs. 16—21, Pl. 2.

Found only in *Octopus vulgaris* Lam. Calotte octamerous; orthotropal in the younger, and many of the medium-sized individuals, but strongly plagiotropal in many of the adults measuring about 2.5mm in length;

obtusely rounded, generally broader than long, and but little wider than the body. The propolar cells differ, as a rule, but little in size from the metapolar cells. The diameter of the propolar set varies but little from that of the metapolar set, except in the plagiotropal calotte. No marked and constant difference in size between the ventral and dorsal polar cells. Parapolar collar adjoined by 2 dorsal and 1 ventral ectodermal cell.

Verruciform cells 0—5.

Total number of ectodermal cells 25.

The description of this species given by Van Beneden, is in the main correct, but, I must add, it seems to have been drawn from a study of few specimens, and certain points have been strongly emphasized which have no specific value, while others appear to have been misapprehended. I have seen calottes similar to the one so accurately and lucidly described by Van Beneden; but this calotte does not, in many respects, represent characters that can be said to hold true of the species. In this calotte, the ventral cells are smaller than the corresponding dorsal ones, and we are told that this feature characterizes the species. Hence it is made the starting-point for general orientation, and for explaining the obliquity and bilateral symmetry of the head.

My observations have convinced me that the size of the polar cells is a very unsafe guide in determining the dorsal and ventral sides of the calotte. In the orthotropal calotte, the only reliable means of orientation which I have been able to discover lies in the relative position of the parapolar and the three proximate ectodermal cells. In the plagiotropal calotte, it is not the size of the cells, but their oblique arrangement that enables one to recognize at a glance the different sides of the head. A comparison of the figures given of this species will show that the obliquity of the head is by no means to be attributed to a difference in size between the dorsal and ventral cells; and further, that it will not do to say that the propolar cells are invariably smaller than the metapolar.

Fig. 17 represents an unliberated embryo, .25 mm in length, seen from the ventral side, as shown by the arrangement of the three cells immediately following the parapolar cells. The inclination of the head and the loosening of the cells are due to the action of acetic acid.

Fig. 16 represents an osmic acid preparation of a young Rhombogen (.6 mm), from the left side; and Fig. 19 (1 mm) another from the same side. There is no noticeable obliquity of the calotte in either of these

cases, and in one (Fig. 19) the propolar cells are fully equal in bulk to the metapolar.

Fig. 20, representing one of the larger individuals, seen from the right side, agrees with VAN BENEDEN's account in showing very little obliquity, and in having the ventral cells smaller than the dorsal; but differs in having the propolar cells decidedly larger than the metapolar. In this latter respect, the figure represents a case which is neither the most frequent nor the most exceptional. This and other like instances noted at the same time, appear to show that the inequality in size between the first and second sets of polar cells has not the uniformity requisite to give it any value as a specific character.

Again, in regard to the obliquity of the calotte, every degree of variation may be found between Fig. 20 and Fig. 21. Fig. 21 shows a perfectly normal and well preserved head of one of the largest individuals (2.5 mm), seen from the dorsal side. Nearly all the longest specimens found at the same time exhibited this extreme obliquity, and some of them a still greater degree. Widely as this calotte differs in its general aspect from that of Fig. 19, a comparison of the two will show that the whole difference may be explained as a forward movement of the dorsal polar cells resulting from the unequal growth of the dorsal and ventral sides of the parapolar cells. As the dorsal propolar cells (*adp*) are carried forward, they must at the same time drop downward in order to preserve their connection with the ventral cells. This will be seen from Fig. 32, a vertical section of a less plagiotropal calotte. If the tilting of the cell *adp* be carried a little further by pushing forward the cell *pdp*, the relative position of the cells would be the same as seen in fig. 21.

2. Dicyema Clausianum E. v. Ben. Figs. 30—34, Pl. 3.

Found exclusively in *Octopus macropus* Risso. Calotte octamerous; orthotropal in the youngest individuals; in the larger examples may be perfectly orthotropal, or more or less plagiotropal; generally broader than long, well marked off from the body.

The propolar cells may be a little smaller than the metapolar, or vice versa. No marked and constant difference in size between the ventral and dorsal cells.

The 2 lateral parapolar cells alternate with the 2 proximate ectodermal cells (one dorsal the other ventral).

Verruciform cells 0—5.

Total number of ectodermal cells 27.

Length of the longest measured, 4 mm; most of the larger specimens measure 2—3 mm.

Van Beneden has given no figures of this species, but has set up the following as differential characters.

1. The calotte is much more oblique than in *D. typus*, and its obliquity is attributed wholly to the ventral cells being smaller than the dorsal.

2. The propolars are conspicuously larger than the metapolars, and in this respect the species is said to differ from all others.

In the earlier cases examined, I found the propolars not larger, but, in many cases at least, smaller than the metapolars (figs. 30 and 33), and was naturally surprised to find my observations thus clashing with those of Van Beneden. I was equally unfortunate in not being able to confirm his statements in regard to the obliquity of the head. Later (Apr. 15) I obtained some material which served to explain, in a measure, these discrepancies. The calottes of three of the larger individuals are outlined in figures 31, 32, and 34. Fig. 34 agrees with statement No. 2, but shows little or no obliquity. In figs. 31 and 32 the obliquity is very well marked, but not so extreme as some cases noted in *D. typus*. The propolars (*avp* and *adp*), seen in optical section (fig. 32), appear larger than the metapolars: but as the latter are considerably longer than the former, the actual difference in volume is certainly not great. A general correspondence between the degree of obliquity and the size of the individuals was observable: but not infrequently individuals of approximately the same length were to be seen lying side by side, some showing no obliquity, others exhibiting it in different degrees. Had my study of this species not extended beyond the examples obtained on this occasion, I should have been able to say that the majority, both among the larger and the smaller, show more or less obliquity; and that in most cases the propolars are nearly equal to the metapolars, in a few even larger. But in my efforts to find specific distinctions that could be said to be decisive in all cases, I have been apprised, over and over again, of the fact, that the obliquity of the calotte and the inequality of its cells are extremely variable, and that the range of variability can seldom or never be ascertained from examples taken from a single *Cephalopod*. This fact makes the task of describing very laborious, since the characters found to hold good in a single case must be revised and supplemented several times before they can be safely regarded as general rather than exceptional.

3. Dicyema microcephalum Whit. Figs. 22—29, Pl. 2.

Occurs only in *Octopus De-Filippi*, Ver. Calotte octamerous, orthotropal, broader than long, often less wide than the body, evenly rounded.

The propolars generally slightly smaller than the metapolars, no difference between the dorsal and ventral cells of the same polar set.

The 2 parapolars followed by 2 dorsal and 1 ventral ectodermal cell.

Verruciform cells 0—6.

Totalnumber of ectodermal cells 26.

Greatest length recorded 2.5 mm.

In the majority of cases I have found the transverse diameter of the head exceeded by the average width of the body, Fig. 22. In one of the larger examples, regarded as a typical representative, the width of the head was .07 mm, that of the body .06—.10 mm.

In many cases the entire ectoderm is charged with granules (»globules refringents« Van Beneden), several cells being more heavily loaded, but otherwise not differing from the rest. These granular cells par excellence, often apparent in the youngest free individuals, are, as a rule, less distinctly verruciform than in other species of the same genus. In one instance the two caudal cells were thus characterized, and much larger than usual, reminding of a condition which prevails among a species found in *Sepia officinalis*.

This species, so far as my observations go, is distinguished from all other species of the genus *Dicyema*, by the permanent and almost perfect quadrilateral symmetry of its calotte (fig. 22—29). The number of cases examined, however, is not sufficiently large to warrant my saying that the species never exhibits the tendency to obliquity noted in all its congeners.

4. Dicyema moschatum. Figs. 1—15, Pl. 1.

Confined to *Eledone moschata* Leach, but not infrequently accompanied by a species belonging to the genus *Dicyemennea* (*D. Eledones*). Calotte octamerous; orthotropal in the embryo, in many of the younger, and in some of the larger individuals; more or less plagiotropal in

most of the adults; conspicuously wider than the body; well marked off; extremely variable in form.

The propolars, as a rule, a little smaller than the metapolars; exceptionally, larger. The ventral cells generally equal to, or larger than, the corresponding dorsal cells. The 2 parapolars followed, in the young, by 2 dorsal and 1 ventral ectodermal cell.

Verruciform cells 2, or none.

Total number of ectodermal cells 24.

Length of larger adults 5 mm, or more.

Of 105 examples of *Eledone moschata*, varying in weight from 15 to 940 grams, 97 contained *Dicyema moschatum*, associated in 14 cases with *Dicyemennea Eledones*; and the remaining 8 contained *D. Eledones* alone. Assuming that these numbers express average relations, it may be said, with approximate accuracy, that in every 100 *E. moschatae*

80 contain exclusively *D. moschatum*,
5 - - *D. Eledones*.
15 - both species.

Thus 95% of the Cephalopods would contain the first, and 20%, the second species.

When both species occur in the same Eledone, they are seldom promiscuously associated. In 3 of the 14 instances recorded, the two species were as completely isolated as of they had been living in different Cephalopods; for each occupied, by itself, one of the two chambers into which the renal sack is divided by a median septum. Where both species were found in the same renal chamber, it was easy to see, in most cases, that they were not indiscriminately intermingled, but distributed in separate groups or colonies, each colony, composed exclusively of individuals of one species, being confined to one, or a few lobes of the spongy renal organ. In some cases only a very few scattered colonies of one species could be found, the other species being represented in great abundance.

I have frequently noticed even where only a single species was present, that certain lobes of the renal organ were beset with exceptionally long individuals, which, judging from the contents of the axial cell as well as the size, represented the earlier settlers. Several times I have met with young Cephalopods in which the entire renal organ, with the exception of one or two lobes, was free from the parasite. These facts indicate that the parasites are not introduced in large num-

bers at any one time; and it does not seem at all improbable that a single individual would suffice to stock a whole renal chamber.

As will be seen from Plate 1, the head of *D. moschatum* exhibits a remarkable diversity of forms, as the products of two ever varying factors, (1) the degree of obliquity, and (2) the relative size and shape of the component cells. So wide is the range of variability, that I was for some time doubtful as to the number of species represented. A comparison of a very large number of individual forms, and a study of the various stages of development, have not brought to light any characters by which one or more of these forms could be specifically separated from the rest. So far as I have been able to ascertain, the whole number of ectoderm cells — barring cases plainly abnormal — is invariably 24, both in the Rhombogens and the Nematogens; and the extremes of obliquity are fully bridged over by intermediate forms. The calottes of the young are always orthotropal, and essentially alike in the form, size, and disposition of the cells. The differences among the adult forms must therefore be of an accidental rather than a specific nature. That the calotte of a single species, within the same species of Cephalopod, should display such a Protean variety of form, is, to say the least, a very interesting fact, and one well calculated to show how extremely unreliable must be the form and relative size of the polar cells in the determination of species. It must be admitted also that this fact gives some grounds to suspect that all Dicyemids of the octamerous type constitute but a single species, the so-called species being only varieties, owing all their distinctive differences to the unlike conditions of life to which they are exposed in their respective hosts. The assumption, however, involved in this view, that these conditions are sufficiently unlike to induce constant differences in size and in the number of cells, as well as characteristic varieties of form, is certainly not supported by the fact that the same species of Dicyema may live, as will presently appear, in two or more distinct species of Cephalopod, without the loss or addition of a single cell, and without any noticeable alteration in length, or in the size, shape, and disposition of the cells. The opinion formely entertained by KÖLLIKER, that all Dicyemids belong to one and the same species, to which the name *Dicyema paradoxum* was given, is still more decidedly disproved by the fact that two entirely distinct species, representing different genera, as I hold, may live side by side in the same species of Cephalopod. VAN BENEDEN's failure to recognize this fact in the case of *E. moschata* must not be taken as an evidence that the differences between the two species are not con-

spicuous; but must be attributed to the mere accident of his not having made analyses in favorable cases. Still, both his descriptions and his figures prove that he had both species under his eye. That his Fig. 1, Pl. 2, was taken from an example of D. moschatum, is unmistakably shown by the contour of the head and the presence of two granular verruciform cells. The enneamerous calotte analyzed in his figures 4 and 5, Pl. 1, is totally unlike that borne by this species, and so conspicuously so that it is difficult to find an ingenuous apology for any confusion in regard to them.

A more detailed description may now be given of the different views of this calotte, seen in Plates 1 and 5. Long before the embryo is ready to abandon its parent, the eight nearly equal polar cells are easy to recognize (fig. 92 Pl. 5). In the fully formed embryo, seen in the lower part of fig. 1, Pl. 1, all the fundamental features of the calotte are clearly defined. At this time its diameter never exceeds the width of the body by more than a trifle, and is generally a little less than its axis. Its outline merges behind in that of the body, and is symmetrically rounded in front. It seldom betrays the tendency to obliquity manifested in later stages. There is no manifest constant inequality in size between the cells of the anterior and posterior set, or between those of opposite sides. The metapolars are cubical, with concave inner and convex outer faces; the propolars are triangular-pyramidal, with one convex outer face and three plane faces by which they are united to each other and to the metapolars. The rounded vertices of the propolars form the anterior end of the embryo; and the middle point, forming the termination of their common line of junction, or axial line, and marked by the intersection of the two dividing planes, may be called the pole of the calotte. The rounded anterior end of the axial cell penetrates a little the basal plane of the four propolars, forming there a small concavity, destined to increase until these cells assume together a watch-glass form.

In fig. 93, Pl. 5, is seen a calotte of a very young individual (.19 mm in length), found free, in which the diameter exceeds the axis, and the propolars are decidedly smaller than the metapolars. Others of a corresponding age were obtained at the same time in which these differences were less marked, or entirely absent. The dorso-ventral symmetry is quite as perfect as the bilateral. The parapolars, meeting in the median line of the dorsal and the ventral side, form thus a complete cervical collar, precisely as in the younger stage of fig. 92, where their lateral angles are much shorter and more obtusely pointed. Of the two

notches formed by the retreating lateral angles, the dorsal is occupied by two ectodermal cells, the ventral mainly by one. The diameter of the cervical collar is fully equal to that of the calotte, and its two cells have about the same length as the other ectodermal cells of the body. The fifth and sixth cells, counting from the hind end of the body, are more granular than the others, and represent the two characteristic verruciform cells of later stages. The cilia of the calotte are more thickly set and shorter than those of the other ectodermal cells. The two calottes just described (figs. 1 and 93) show about the range of variability in these early stages, and their differences recur between the embryo and the parent in fig. 1. From these forms, figures 2 and 3, taken from two Rhombogens of equal length (1.20 mm), differ in several noteworthy respects. The diameter of the head is much greater than that of the parapolar collar, and the metapolars flare a little at their hind margin. In fig. 2, seen from the ventral face, the dorsal metapolars appear to be a little larger than the ventral; but most of the oblique calottes agree with fig. 3 (seen from right side) in having the ventral metapolars decidedly larger than the dorsal. The same inequality holds true, with less constancy, between the ventral and dorsal propolars (figs. 4, 5, 6, 9). The difference in size between the propolars and the metapolars is most frequently in favor of the latter.

One of the most uncertain and variable features of the adult calotte, is its degree of obliquity, which can be said, only in a most general way, and by allowing a very liberalmargin for exceptions, to increase with age.

A dorsal and a profile view of two strongly plagiotropal calottes, from individuals of the same length (3.50 mm), are given in figs. 5 and 9. In both, the four propolars have a ventral position; while the ventral metapolars are much elongated, and so curved that one end is ventral, the other dorsal, and the middle portion lateral. In bending towards the dorsal side, they are at the same time prolonged forward, and thus describe a curve of double curvature. This peculiarity repeats itself pretty constantly in the most oblique calottes, and helps to explain how the obliquity arises.

The forms of this heteromorphous calotte, seen in Plate 1, first as hemispherical (fig. 1), then pyramidal (fig. 2), galeate (fig. 3), globose (fig. 7), clypeate (figs. 5 and 9), campanulate (fig. 6), etc., although far from being exhaustive, are at least sufficient to convey some notion of its Protean character, and to illustrate some of the principal lines of form-variation. They were all taken from well developed and well

preserved individuals, and, with perhaps the exception of figs. 6 and 8, represent forms of frequent occurrence.

It will be seen from these figures that the form assumed by the cephalic end of the axial cell is essentially the same for both Rhombogens and Nematogens. Van Beneden found that the Rhombogens are as a rule shorter than the Nematogens, and this agrees with my observations. But the Rhombogens sometimes attain a length that is seldom exceeded by the Nematogens. One Rhombogen, taken up from the renal fluid by the aid of a pipette, and measured immediately after killing with acetic acid, was 5.75 mm long. I have recorded no cases in which Nematogens measured more than 6 mm.

The shorter individuals often show the greatest width just behind the head (fig. 13), tapering from this point slightly towards the hind end; while in the larger specimens the width is more uniform, being .08—.10 mm for a length of 5 to 6 mm. Two large verruciform cells, filled with refractive globules, and located in the posterior half of the body, at opposite sides, are quite constant in this species; but they are sometimes missing both in the younger and older forms.

5. Dicyema truncatum Whit. Figs. 51—59, Pl. 4.

Occurs in *Sepia officinalis* Lin. (here usually associated with *Dicyemennea gracile*), in *Sepia elegans* Blain., and in *Rossia macrosoma* Delle Chiaie. Calotte octamerous, small, discoid, capping the broad truncated ends of the parapolars, which form the greater part of the head; its face somewhat convex in the young; occasionally so in the adult.

The nearly equal propolars (2 dorsal and 2 ventral) much smaller than the metapolars, and encircled by them. The equal metapolars alternate with the propolars, two being lateral, one ventral, and one dorsal. The diameter of the parapolar collar greater than that of the body, or of the polar cap. Ectodermal cells disposed in alternating pairs. Two terminal cells are very constantly charged with refractive granules, and more or less pyriform.

Total number of ectodermal cells 22.

Length .50—.75 mm.

Dicyema truncatum is a very well characterized species, and differs more widely from *D. gracile*, with which it is commonly associated

in *Sepia officinalis*, than from either of the other species thus far described. Under the persuasion that these two species were one and the same, Van Beneden was led to attribute to a calotte belonging to *D. truncatum* characters that are wholly foreign to it. He affirms that this calotte is composed of nine cells, and in his fig. 6, Pl. 1, he has actually delineated this number of polar cells, which certainly exceeds by one the number placed there by nature. This species bears an octamerous calotte, while that of the associate species is enneamerous: and this fact alone proves that their separation is well founded. A glance at figures 48—50 (*D. gracile*) and 53—59 (*D. truncatum*), will make it evident that the two species are quite distinct in many other respects. They are distinguished by the total number of cells, by the length and general appearance of the body, by the form and the character of the two caudal cells, by the shape of the cephalic enlargement, and by the share taken in its formation by the parapolars. Most of the distinctions seen in the adult forms are also to be found in the young, and even in the unliberated embryo, so that there remains no ground for the supposition that *D. truncatum* is only a younger form of *D. gracile*.

Van Beneden describes the »renflement céphalique«, represented in the figure just referred to, thus:

»In *Dicyemina Köllikeriana* of the Cuttle-fish there are nine polar cells disposed in two sets. These cells are very small, compared to the polar cells of *Dicyema* and *Dicyemella*. They form together a very granular, opaque body, capping the anterior extremity of the endodermal cell. But this coiffe is very eccentrically placed in the cephalic enlargement (voir pl. 1, fig. 6). It is strongly inclined toward the ventral side. In young individuals, the nine polar cells forming this coiffe are placed in the same oblique plane with respect to the axis of the body. This plane looks downward and forward, when the organism is placed in a normal position. The head appears cut in front by an oblique truncation. The first set of polar cells comprises four conoid or pyramidal cells much smaller than those of the second set; and two of these are smaller than the other two, and turned toward the ventral face. In the second set one counts five prismatic cells placed, with respect to one another and to the central polar cells of the coiffe, as in the species of *Eledone moschata*.

As the individual increases in size, the polar coiffe becomes relatively more extended and its characters so modified that it becomes more like the polar organ of the Poulp and Eledone.« (p. 18 and 19.)

From this it appears that Van Beneden regarded *D. truncatum* as

the young, and *D. gracile* as the adult, of *Dicyemina Köllikeriana;* and he very naturally inferred that the nine polar cells of the mature forms must be present in the so-called »young individuals«. Bearing this fact in mind, it is easy to see what parts of the above description were actually drawn from a study of *D. truncatum*. The smallness and obliquity of the calotte are attributes belonging to this species, while the number and, in the main, the disposition of its cells are applicable only to *D. gracile*.

The description of the parapolar cells (p. 20—21) accords nearly with my own observations. »These cells are two in number, and differ from each other neither in form nor volume, nor in any other character. They are distinguished from the ectodermal cells of the trunk by their form and contents. In well developed individuals, they are nearly elliptical in optical section (pl. 1, fig. 14). . . . . The small axis of the ellipse is about equal to three fourths of the large axis. The contents of these cells is much darker than that of the ectodermic cells of the trunk; it is finely granular, but never charged with the refractive globules which are almost constantly met with in the other cells of the ectoderm, and which, by accumulating, produce warts (verrues).

These two parapolar cells are placed on the lateral faces of the head. They meet along the median dorsal and the median ventral line, thus forming a collar, through which passes the endodermic cell. Each constitutes a moiety of the ring. This ring is much shorter at the dorsal and the ventral line of junction than at the sides of the head. The ectodermal cells of the trunk, which immediately follow the parapolar cells, end in a point between the latter, but never reach the cells of the polar coiffe.«

I have never observed the strong contrast here said to exist between the contents of the parapolars and that of the other ectodermal cells; and I should say that the form of the parapolars, seen in optical section, is triangular rather than elliptical.

With reference to the inclination of the calotte, I do not see how Van Beneden's figure 6, Pl. 1 can be reconciled with his statements. Judging from the position of the parapolars, this figure represents the head as seen from the right, or left side; but how, in either case, can the calotte be said to incline to the v e n t r a l face? Again, taking the size of the propolars as a means of orientation — allowing the ventral cells to be smaller than the dorsal — the calotte appears to be seen from the dorsal face, and to be inclined towards this face, all of which is equally contradicted by the position of the parapolars. As I have

never found any uniform inequality such as Van Beneden insists on between the dorsal and ventral polar cells, I am compelled to determine the direction of inclination by reference to the parapolars, and therefore to regard the figure as seen from one of its lateral faces. If I am correct in this, the figure represents a case of obliquity fully analogous to that seen in fig. 57, showing that it may occasionally be dextral, or sinistral, instead of ventral. This irregularity in the direction of the inclination, of which I have seen a considerable number of instances, and the inconstancy of its occurrence presently to be noticed, lead to the conclusion, already expressed with reference to some of the foregoing species, that very little importance can be attached to this phenomenon as a specific character. In order either to remove or explain the incongruity between this opinion and that emitted by Van Beneden, I have made repeated examinations with special reference to this point. The result has been that I have found it necessary, first of all, to explain the incongruities between my own observations; for what was recorded as the rule one day, appeared to be the exception the next. After examining carefully a large number of individuals obtained from a Cuttle-fish Mar. 14, I made the following note:

»As a rule the inclination is hardly to be recognized, and I doubt if it be a normal feature.«

A later note reads thus:

»I find that there is a plain obliquity in many heads; but it is by no means constant, nor can I say that it exists in the majority of cases.«

That the same species of Dicyema varies sometimes considerably from one Cephalopod to another, is a fact which has forced itself upon my attention in nearly every species that I have examined; and it is more on account of its general importance than its special significance that I have gone into details with the ease in hand. According to my observations, then, obliquity of the calotte is exceptional in some cases, and more or less general in others; it may be dextral or sinistral, but is more frequently ventral.

With reference to the arrangement of its cells, this calotte differs in one particular from other octamerous calottes. The entire difference, however, may be explained as the result of a rotation of the metapolar disc on its axis through 45°, the propolar disc maintaining its original position. This rotation of an octant makes two of the metapolars lateral, one dorsal, and one ventral (figs. 58, 54, 57). The arrangement of parts thus becomes similar to that of a flower, the perianth of which consists of two cruciform whirls of leaves alternating with each other.

This alternate order of the polar cells is quite distinct from the opposite order seen in all the other species of the genus, and might perhaps serve as a basis for the formation of a new genus; but as I find only one species thus marked, there seems no urgent necessity, at present, for burdening science with a new name.

The calotte is nearly hemispherical in the embryo, and may be said to form the entire head. These relations are very seldom seen in the adults (fig. 59), the calotte becoming, as a rule, more or less flattened with age, owing to the fact that its growth does not keep pace with that of the parapolars (figs. 53, 54, 57).

Van Beneden estimates the number of ectodermal cells at 25. Although I am certain that he has counted one too many in the calotte, I am not equally so that he has made an error of two in the body. While I have never succeeded in finding more than 22, in specimens taken from *S. officinalis*, *S. elegans*, or *Rossia macrosoma*, I have seen cases with only 20, and one of these is given in fig. 53. But such cases are exceedingly rare, and the still greater variations, such as van Beneden has described (see his fig. 8, Pl. 2), are unquestionably to be regarded as abnormal. The number 25 agrees neither with that which I regard as normal for *D. truncatum*, nor with that of the associate species, *D. gracile*.

The caudal cells are generally two and symmetrically placed; but cases have been met with in which three of the terminal cells were charged with granules, all presenting the same outward appearance and forming together a trilobate ending. In some instances only one of the two normal caudal cells were loaded with granules; and frequently both cells are very small and inconspicuous (fig. 52).

I have always found this species very abundant in *S. officinalis*. By its short and rhabdoidal form it is easily distinguished from the elongated and slender *D. gracile*, which occurs in much smaller numbers. That this species is identical with that found in *Rossia macrosoma* and *Sepia elegans* is, to my mind, as certain as it can be, so long as our knowledge of the cycle of life of these animals remains incomplete. In the first case of *Rossia* that was examined for the purpose of comparison, I noticed one or two peculiarities which I had not recognized in specimens from *S. officinalis*. The calotte formed, in many individuals (fig. 59) a large portion of the head; and the entire ectoderm, with the exception of the finely granular polar cells and the large caudal cells, was charged with coarse shining granules of an elongated angular form. In every other respect, the specimens agreed precisely

with D. truncatum of *S. officinalis*. Before these differences could be accepted as conclusive evidence of a new species, it was necessary to know if they were constant. Further examinations proved the contrary. In the second case recorded, the difference in the granulation of the ectoderm was absent; and the calotte, if a little larger than the average in *D. truncatum* of *S. officinalis*, agreed so nearly in this respect and so perfectly in every other particular, that I felt compelled to abandon the idea of attaching a specific value to these distinctions.

Dr. Foettinger (29), in a recent paper on some new Infusoria, found in the renal organ and the liver of certain Cephalopods, incidentally remarks that the Dicyema of *Sepia elegans* is a new species, which he proposes to describe in a future communication. I have examined five *S. elegans*, and have found Dicyema in every case; and I have failed to detect any constant difference between them and *D. truncatum* of *S. officinalis*. The differences which I have noted in individual cases have not proved to be constant, nor have they been either greater or more numerous than those observed in specimens obtained from different *S. officinales*. The chief difference noted concerned the caudal cells, which were prevailingly small and otherwise not so well characterized as they generally are in individuals from the Cuttle-fish. But as a considerable number of individuals did not differ at all in this respect from the average form met with in the last named Cephalopod, the difference can not be regarded as anything more than a tendency to variation induced by the somewhat unlike conditions of life offered by the two different hosts.

6. Dicyema Schulzianum E. V. Ben.

Found in Sepia biserialis Den. de Mont.

Unfortunately I have not met with individuals of this species sufficiently numerous and well preserved to admit of accurate analysis, and have therefore but little to add to the very brief account given by Van Beneden. His statements are, that the parapolars take part in the formation of the head, as in *D. truncatum*; the calotte is larger than that of *D. truncatum*, forming a large part of the cephalic enlargement in adults, and the whole of this enlargement in the young; the metapolars are quite different from the propolars. Nothing is said in regard to the number of cells in the calotte, or the total number of ectodermal cells. I am quite certain

that there are eight polar cells; but cannot affirm positively that they are arranged precisely as in *D. truncatum*. I have never seen any individuals with large caudal cells. The length of this species agrees nearly with *D. truncatum*.

7. Dicyema macrocephalum E. V. Ben. Figs. 35—39, Pl. 3.

Found only in *Sepiola Rondeletii* Gesner.

Calotte octamerous; almost constantly plagiotropal; presenting a flattened, more or less circular, or somewhat quadrilobate face; broader than the body, and expanding into a free plate-like margin. The sub-equal propolars form the central portion of the cephalic plate, and the slightly unequal metapolars its free margin or rim. Each set of polar cells presents two ventral and two dorsal cells, the cells of one set being opposite the corresponding cells of the other set. The two lateral parapolars, somewhat thickened at their junction with the calotte, are followed by three ectodermal cells — 2 dorsal and 1 ventral.

The ectodermic cells disposed in 6 alternating sets of 3 cells each, interposed between the parapolars and the 2 caudal cells.

Verruciform cells 0—5.

Total number of ectodermal cells 30.

Length 5—7 mm.

Van Beneden has represented the calotte of this species turned 45° out of its natural position (compare his figs. 3 and 5, Pl. 2, with my fig. 37, Pl. 3), and this displacement introduces features that appear to separate it from all other octamerous calottes. Again, he is equally unfortunate in affirming that there are four parapolar cells instead of two as in all other Dicyemids. If Van Beneden were correct in these two points, I should be free to admit the necessity of forming a new genus; but he is certainly wrong in both, and the error may probably be attributed to a too hasty examination of unfavorable examples.

I have seen cases in which the calotte appeared to be in precisely the same position Van Beneden has given it; but a study of the younger stages and a comparison of a large number of adult forms have shown that in each set of polar cells two are ventral and two dorsal. To make certain of this point, I have examined specimens alive, and

moving freely in the renal fluid. In this condition it is easy to see, in a lobed calotte resembling that in fig. 35, that two lobes are ventral and two dorsal. When specimens are killed in acetic acid, or by any other means, and covered by a glass, the calotte is exposed to pressure, unless protected by supports placed under the cover. Such pressure is all that is required, especially with individuals lying sidewise, to induce appearances of the same nature as Van Beneden has described.

With respect to the number of parapolar cells, it is easy to be misled, particularly in adult forms. The two lateral angles of the parapolars (fig. 36) are turned strongly toward the ventral side, and the dorsal notch left between them is filled by the fore ends of two ectodermal cells, which extend far forward, but never reach — so far as I have observed — the polar cells. It is these two cells that, added to the two lateral parapolars, make up the four parapolars of Van Beneden's description. It is not always easy to see, in the larger examples, the anterior limits of these two cells; but the development, and the whole series of younger stages, bear witness to the fact that this species agrees with all others at present known, in respect to the number of its parapolar cells. In fig. 38, from a young individual only .7 mm long, a complete parapolar collar is seen, formed of two lateral halves, joined in the median dorsal and median ventral line. From the same figure it is plain that there are two dorsal and two ventral metapolars. The propolars, not represented in this fig. lay in the same plane with the metapolars, not projecting beyond them, as seen in the younger form of fig. 39. I think the parapolars never take so conspicuous a part in the formation of the head as Van Beneden has represented. Cases similar to his figures have been seen, but they appeared to me to present unnatural forms due to swelling.

The number of verruciform cells varies from 0 to 5. They may be quite conspicuous, but I have never seen instances of their projecting strongly beyond the other ectodermal cells.

With the above exceptions, Van Beneden has given a very accurate description of the calotte: »The calotte is here a cellular plate formed of flattened cells. This plate is very obliquely placed at the anterior extremity of the endodermal cell; it looks toward the ventral side. In well developed individuals it is sometimes plane, but more frequently concave (?). In all young individuals it is convex. It is formed of eight polar cells disposed in a central set of four cells and a peripheral set of the same number. When the calotte is seen from the face, the central cells show a triangular form; the peripheral cells form

around the central zone a ring composed of four segments. Each of these segments is a cell.«

8. Dicyemennea Eledones Wag. Figs. 44—47, Pl. 4.

Found in *Eledone moschata* Leach (usually in company with *Dicyema moschatum*) and *Eledone Aldrovandi* Delle Chiaie.

Calotte enneamerous, conoidal, permanently orthotropal, wider than the body.

The 4 equal triangular-pyramidal propolars always much smaller than the 5 sub-equal prismoidal metapolars. The propolars are 2 dorsal and 2 ventral; the metapolars 3 dorsal (1 median and 2 lateral) and 2 ventral. The cephalic enlargement formed chiefly by the calotte, but, to some extent, by the 2 lateral parapolars. The ectodermal cells, beginning with the parapolars, arranged in 7 alternating pairs.

Verruciform cells none.

Total number of ectodermal cells 23.

Length 5—7 mm.

The occurrence of this species in *Eledone moschata* has already been detailed in the account of *D. moschatum*. I have found it in ten of the twelve *E. Aldrovandi* which I have examined, and always unaccompanied, except by *Benedenia elegans* or *B. coronata*. Thus the frequency of its occurrence may be estimated at 6 : 30 in *E. moschata*, and 25 : 30 in *E. Aldrovandi*.

I have been unable to detect any peculiarity in individuals obtained from one host not found in those from the other; and in no instance have I met with anything that could raise a doubt as to their specific identity.

As this species is found in at least two different Eledones, and has not thus far been found in any other genus of the Cephalopods, the name *D. Eledones* Wagener, is very appropriate, and for this, as well as other reasons before mentioned, has been preferred to *D. Wageneri*, proposed later by Van Beneden.

In gracefulness of form and transparency this species is excelled by none; and in length, by *D. macrocephalum* alone. In these respects it agrees nearly with *D. gracile* of *Sepia officinalis* (fig. 48).

The form of the calotte, which differs in a most marked manner

from any thus far described, is shown in figs. 44, 45, and 47, Pl. 4. The difference in general aspect between the fully developed embryo (fig. 47) and the adult (figs. 44 and 45) is trifling compared with what we have seen in most species of the octamerous type.

The inequality between the axis and the diameter of the calotte is greatest in the embryo, while the disparity in volume between the propolars and the metapolars is greatest in the adult, all of which is due to the fact that the metapolars enlarge at a much more rapid pace than the propolars.

With respect to the number and the disposition of the cells (compare fig. 46 which represents both ranges in optical section), I find Van Beneden's statements perfectly correct: »The polar cells of the first range are four. . . . . In the second range there are five cells, and these are much larger than those of the first range, and their aspect is very different. Of these five cells two are ventral. Of the three dorsal, one is median and two lateral.« p. 18. I can not say that the two ventral propolar cells are uniformly smaller than the two dorsal, as Van Beneden maintains. The metapolars are sometimes equal in length (fig. 44), and sometimes the three dorsal cells are a little longer than the two ventral; but in either case the calotte maintains the same orthotropal position. In some cases I find the calotte almost free from granules, in others the propolars more granular than the metapolars, and again both sets more granular than the body.

In fig. 47 (seen from the side) the parapolars are lateral and the caudals dorsal and ventral; now if the alternation in the successive pairs was always one of 90°, the caudals would agree with the parapolars in being lateral. As the figure shows, the alternation follows a sinistral spiral line which sweeps an arc of 90°, in passing from the anterior to the posterior end of the body. The same peculiarity is often more manifest in adults, and is not confined to this species. In nearly all long Dicyemids the elongated ectodermal cells show, individually, a more or less marked spiral curvature.

9. Dicyemennea Mülleri Clap.

This species was found by Claparède, Lachmann, and Joh. Müller in *Eledone cirrosa* on the coast of Norway. Neither the figures nor the descriptions given by Claparède and Lachmann enable us to say with certainty that this species is not the same as the one just described. It is said to vary much in form. »Sometimes it is a long filament, every-

where of equal breadth; sometimes its anterior portion is very broad compared with the posterior part, which is slender and much elongated, resembling then a tadpole; and, again, the body is, so to speak, contracted, comparatively short and thick.« 6, p. 202. The number of the polar cells (»plaques«) was not ascertained, and hence its generic position remains problematic. The various forms of the body give some ground for thinking that this species is distinct from *D. Eledones*, while the form of the calotte excludes it from the genus Dicyema, and makes it probable that it has been correctly placed in the genus Dicyemennea.

10. Dicyemennea gracile Wag. Figs. 48—50, Pl. 4.

Found only in *Sepia officinalis*, always in company with *D. truncatum*. Calotte enneamerous, globose, orthotropal, wider than the body and well marked off.

The polar cells agree in number and disposition with *D. Eledones*. The propolars always much smaller than the metapolars.

The head formed exclusively by the polar cells.

The 2 lateral parapolars scarcely differ in thickness and general appearance from the ectodermal cells of the trunk.

Ectodermal cells, thin, their number and arrangement the same as in D. Eledones.

No verruciform cells.

Length 4—6 mm.

This species has been found in every *S. officinalis* that I have examined, but much less abundant than the companion species. It is generally distributed in colonies in the manner described under *D. moschatum*.

The wide difference in form and aspect between this species and *D. truncatum*, with which it is associated, is made apparent by figs. 48 and 53, Pl. 4; and the equally conspicuous differences in the form and composition of the calottes may be seen by comparing figs. 49—50 with 54—59.

The differences between this Dicyemid and *D. Eledones* are of such a superficial nature that I cannot regard them as conclusive evidence that the two forms are specifically distinct; still they are sufficiently obvious and general to justify a provisional separation.

The points of agreement are:

1. Total number and arrangement of cells.
2. Form and general aspect.
3. Number and disposition of the polar cells.

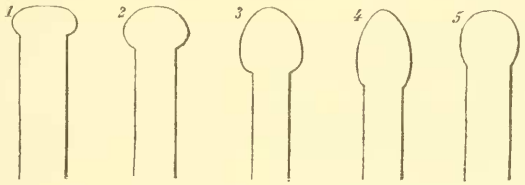
They differ, as a rule, in the following particulars: In *D. gracile*

1. the head is smaller and more spherical.
2. The fore ends of the metapolars are thicker than the hind ends.
3. The parapolars are not thickened at their junction with the calotte, and consequently take no part in forming the head.

The more common form of the head is seen in fig. 49, and an exceptional form in fig. 48.

The following forms (1—5) were all sketched from large individuals found in the same *Sepia*.

Such forms as 1, 2, and 5 have never been seen in *D. Eledones*.



Since this chapter was written, a second paper by Van Beneden has appeared (No. 2), in which he describes two new genera, Conocyema and Microcyema. As these forms are supposed to represent a family distinct from that of the »true Dicyemids«, it does not appear to be necessary to make any changes in the foregoing pages.

Chapter II.

Reproduction.

The two kinds of embryos produced by the Dicyemids were seen and described by Erdl nearly forty years ago, but were supposed by him to represent different stages in the development of one and the same individual.

Kölliker was the first to show that these embryos are two entirely distinct forms, arising from two different germs, which pursue two unlike courses of development while within the parent body. He ascertained further that the two sorts of embryos, which he distinguished as

infusoriform and vermiform, are not found together, but each by itself in different individuals.

These conclusions were confirmed by the researches of Ed. Van Beneden, who followed the development of both forms with much more care and success than had been done before. Further than this, Van Beneden drew a sharp line of distinction between individuals bearing infusoriform and those bearing vermiform embryos, designating the former as Rhombogens, the latter as Nematogens. Beyond the fact of their dimorphic progeny, already established by Kölliker, the following differential characters were declared to exist:

Rhombogens.	Nematogens.
1. Comparatively short and thick.	1. Comparatively long and slender.
2. Axial cell correspondingly broader, having a rounded termination in the head.	2. Axial cell narrower, and tapering to a point in the head.
3. Polar cells more flat.	3. Polar cells thicker.
4. Number of ectodermal cells variable, generally less than in Nematogens.	4. Number of ectodermal cells constant, and often greater than in Rhombogens of the same species.
5. Germ-cells relatively small (0.012—0.014 mm); formed endogenously in the reticulum of the axial cell.	5. Germ-cells large (average 0.021 mm) formed endogenously in special cells (»germigens«) lodged in the axial cell.

In view of the differences here enumerated, and the concurrent testimony of Kölliker and Van Beneden to the effect that the two kinds of embryos are never found in the same individual, it may, at first sight, appear incredible that the Rhombogen and the Nematogen are nothing more nor less than two consecutive phases in the same individual cycle of life. The presentation of the evidence on which this assertion rests, will show that the distinctions before named, with some slight modification, are not irreconcilable with it.

The fact that these creatures can not be kept alive for more than a few hours, precludes of course the possibility of tracing the transition from one condition to the other by continued observation on a single individual.

The indirect character of the evidence as well as the peculiar nature of the phenomenon itself calls for a detailed statement of observations and the inferences drawn from them.

Van Beneden's studies led him to think it probable that Rhombogens and Nematogens are heterogeneous forms, permanently distinct from each other. But he remarks: »I do not know what determines the

difference between the Nematogens and the Rhombogens. I do not know if an individual, after having produced and discharged vermiform embryos, can, arrived at a certain age, become modified and produce infusoriform embryos; or if the Nematogens are originally distinct from the Rhombogens. The latter opinion seems to me more probable; but if it be so, what causes one vermiform embryo to become a Nematogen, another a Rhombogen?« (1, p. 68). To these questions Van Beneden's investigations supplied no answers.

The first thing in the course of my own observations to draw my attention to the question of the relationship between the two sorts of Dicyemids, was the occasional finding of a Cephalopod in which, so for as could be ascertained, only Nematogens, or only Rhombogens, were present. If, as Van Beneden's work seemed to show, both kinds have the same course of development, i. e. both arise from similar vermiform embryos, how is it that in one Cephalopod all, or nearly all, are Rhombogens, in another, all, or nearly all, Nematogens? If within the renal organ these parasites multiply only by v e r m i f o r m embryos (1, p. 68), how is their propagation provided for in those cases where all are Rhombogens?

In Van Beneden's first paper (1), I find no allusion to these points: but in his last (2, p. 209) occurs the following: »I have succeeded no more than G. Wagener in finding typical infusoriform [embryos] in the spongy bodies of the Cuttle-fish of the Mediterranean. It was in vain that I sought for them during the months of August and September, both at Villefranche and at Trieste. But I was more fortunate in examining Cuttle-fishes from the North Sea, received during the months of October, January, and February. All enclosed at least as many Rhombogens as Nematogens; and the Infusoriform [embryo] of *Dicyemina Köllikeriana* (*Dicyema gracile* G. Wagener) does not differ perceptibly from that of the other Dicyemids. On several occasions, my father has observed the Dicyemids of the Cuttle-fish: I find in his notes and sketches that he has always met with the Infusoriforms in great abundance; it is a Rhombogen of *D. Köllikeriana* that he has figured and designated under the name *Dicyema Krohnii*, in his 'Commensaux et Parasites'. How does it happen that neither Wagener nor I have found the Infusoriform of the Dicyemina of the Cuttle-fish of the Mediterranean? That is a question to which I can not reply, unless by the wholly gratuitous supposition that the Rhombogens appear perhaps only at certain times of the year.«

In the same number of the 'Archives de Biologie', in drawing

a parallel between the *Dicyemids* and the *Orthonectids*, JULIN remarks: — »It follows from the observations of EDOUARD VAN BENEDEN that in the case of the Dicyemids the same host generally incloses, all Nematogenic or all Rhombogenic individuals; It is only very seldom that one finds the same host infested with both forms. A fact quite analogous holds true of the Orthonectids. Here we find always two female forms in the same host; but most frequently one finds exclusively males or females in the same Ophiurid.« (21, p. 44.)

I have introduced the statements of JULIN, not because I find them — so far as they concern the Dicyemids — warranted by anything VAN BENEDEN has yet published, but because they claim to be authorized by his researches.

The instances in which the Cephalopod incloses only one of the two forms of Dicyemids are, according to my observations made in the months from December to April inclusive, much less frequent than those in which both are found together. It should be remembered moreover that instances of the first kind may be considerably less frequent than they appear to be; for it is extremely rare that one can make such a thorough examination of the renal organ that the phrase »not present« may be safely substituted for »not found«.

Young stages of Rhombogens and Nematogens are liable to be confounded; and, hitherto, transitional forms have been entirely overlooked. It sometimes happens that the individuals of one kind are confined to one, or at most two or three lobes of the renal organ, and are thus easily overlooked. In view of these sources of error, I am compelled to regard with suspicion those cases of isolated occurrence of Rhombogens or Nematogens which were recorded before I became fully aware of the ways in which an examination might miscarry. For the same reason and others presently to be mentioned, VAN BENEDEN's failure to find Rhombogens in the Cuttle-fish of the Mediterranean can only be accepted as doubtful evidence of the absence of such Dicyemids. That they are to be found throughout the five months from December to April is perfectly certain.

There are two questions to be answered with reference to the distribution of Rhombogens and Nematogens:

1. Do they ever occur isolated?
2. Is their occurrence seasonal, or in any sense periodical?

The first question may, with considerable certainty, be answered in the affirmative; and the observations on which this answer is based tend to show that the occurrence of the two forms is not regulated by

seasonal influences. Although, so far as I can judge, the cases in which it can be said with a very high degree of probability that only Rhombogens or only Nematogens are present are comparatively few, still we cannot deny their importance. They can not of course be accepted as proof that one form passes into the other, but they are more easily reconciled with such a transition than with any contradictory hypothesis that has yet been offered. My observations on these points have been made chiefly on the parasites of *Eledone moschata*, and here I find only one case recorded in which Rhombogens alone were found, and eleven in which Nematogens were similarly isolated. Many cases were noticed in which both kinds were about equally numerous, but a much larger number of cases in which there was a more or less decided numerical preponderance in favor of one or the other form.

In the early part of March I happened to receive only small examples of *Eledone moschata* for several days in succession. In all I found the Nematogens very abundant, and in some, Rhombogens appeared to be wholly absent. The possibility of a seasonal occurrence of the Rhombogens, as suggested by Van Beneden, then presented itself: but this idea was not sustained by subsequent examinations. I soon recognized a remarkable degree of correspondence between the size of the Cephalopod and the numerical relations of the two kinds of Dicyemids. Large Eledones invariably inclosed Rhombogens, often in much greater abundance than Nematogens; while very small Eledones showed obverse relations, sometimes furnishing only Nematogens. I made numerous examinations during March and April with special reference to this point, and a comparison of the results obtained from Eledones weighing from 15—50 grams with those from Eledones weighing from one to several hundred grams, show that the correspondence above stated is not an exception but an invariable rule. As examples the following cases are given.

Apr. 24. *E. moschata* 25 grms. *Dicyema moschatum*; mostly Nematogens, but a considerable number of Rhombogens among the smaller individuals.

\- - - 24 - *Dicyema moschata*; all Nematogens.

\- 25. - 15 - One tuft', or colony of large clear Nematogens (*D. moschatum*) without verruciform cells; a large tract of short Nematogens of the same species, mostly with two verruciform cells. In another part of the renal organ, long Nematogens (*Dicyemennea Eledones*). No. Rhombogens.

\- 24. - 185 - Mostly Rhombogens (*D. moschatum*). A few Nematogens inclosing two or three embryos

and many germ-cells; a larger number of individuals containing no embryos, but crowded germ-cells, with 1 to several large free nuclei in th axial cell.

Apr. 26. *E. moschata* 940 grms. *D. moschatum*, abundant, mostly Rhombogens. Only a few Nematogens containing vermiform embryos. A considerable number of young Rhombogens, and individuals inclosing only crowded germ-cells. Most of the Nematogens and those containing only crowded germ-cells showed several large free nuclei in the axial cell.

With the exception of the last *Eledone*, which was the largest that has been seen for several years at the Zoological Station, the larger specimens have weighed from one to four hundred grams. Although this was an exceptionally large *Eledone*, the Dicyemids of its renal organ differed in no marked respect from those of the Eledone weighing 185 grams. These five cases illustrate a fact attested by numerous comparisons in March and April, and, so far as my notes and memory inform me, by all observations of a prior date. A series of examinations extending through an entire year are required in order to settle beyond dispute the question of seasonal influence on the occurrence of the two kinds of Dicyemids; but it seems to me quite certain, at least in the case of *Dicyema moschatum*, that their relative abundance from December to April inclusive is regulated by influences of another nature. What is the meaning of the parallel between the abundance of the Rhombogens and the size of the Cephalopod? Are the conditions of life under which these parasites live in mature Cephalopods unlike those prevailing in immature individuals? and if so, is there any causal relation between these unlike conditions and the appearance of Rhombogenic Dicyemids?

Or is the phenomenon a cyclical one, wholly independent of the age of the Cephalopod, although showing a certain degree of correspondence to it? and in this case, is the Rhombogenic condition an integrant part of the life-history of all Dicyemids? or only a phase in the life of some or all individuals of certain generations? Is it an introductory, or a concluding phase?

As the succeeding pages will show, I have succeeded in obtaining answers to only a few of these questions.

The Axial Cell.

At a very early date in my study, I became aware of the fact that the axial cell is often plurinuclear, not only in Rhombogens but also in

Nematogens. That the axial cell of a Nematogen sometimes incloses not less than nine large free nuclei, at other times only one, seems to have been entirely overlooked by Van Beneden. In the case of the Rhombogens, on the other hand, the plurinuclear condition was recognized and partially accounted for. The occurrence of the same condition in Nematogens remained a puzzle for a considerable time, as I was unable by comparison of different stages of these individuals to obtain any clue to the origin of several nuclei. In the Rhombogens, however, I have been able to trace in a very complete manner the development of this feature; and I now regard its occurrence in Nematogens as one of the most conclusive evidences of a transition from one form or condition to the other.

A curious fact with respect to the number of these free nuclei may here be mentioned, — a fact which can be explained on no other hypothesis than that of a transition from the Rhombogenic to the Nematogenic condition. In Rhombogens the number may be either odd or even; in Nematogens it is invariably odd. The explanation of this peculiar difference will be seen when we come to trace the origin of the nuclei in the Rhombogens.

The differences in the form of the axial cell are neither uniform nor permanent. Under the head of differential characters, Van Beneden states that the anterior extremity of this cell takes a rounded form in Rhombogens, and a pointed form in Nematogens. That this distinction is not uniform is proved not only by my figures, but also by those of Van Beneden.

The Origin of Free Nuclei in the Axial Cell.

In the fully formed embryo of *D. moschatum* (fig. 1, Pl. 1), the axial cell shows a single central nucleus (*ne*) and two primary germ-cells (*pg*).

This can not be said to be the original nucleus of the axial cell, as supposed by Van Beneden, since it is only one of the division-products of that nucleus, as will be shown in discussing the origin of the germ-cells.

Although it is not to be confounded with the nuclei which appear later in the axial cell, I have not always been able to distinguish between them, and therefore, as a matter of convenience, include it among the number of free nuclei, without intending thereby to express any opinion on its relation to the axial cell.

Sometimes this nucleus maintains its central position in the adult,

and is easily recognized from other nuclear bodies by its greater volume and more elongated form. But it frequently happens that all these means of recognition are lost: its position may be in the head or the tail, or in any part of the body; it may exhibit a spherical form and differ so little in size from neighboring nuclei that discrimination becomes impossible.

Both Rhombogens and Nematogens arise from vermiform embryos like that seen in fig. 1; and it is impossible at this stage to predict which form will be brought out by subsequent development.

Shortly before, or soon after the embryo escapes from the parent, we find four germ-cells — two before and two behind the nucleus (fig. 95, Pl. 5). Next comes a stage of eight germ-cells arranged in a single loose row along the middle of the axial cell — four before and four behind the central nucleus (fig. 100, *ne*). It is about this time that the first indications are given of the kind of reproductive activity the individual is destined to display. The continuation of the same mode of multiplication leads to the Nematogenic condition, represented by more or less numerous germ-cells scattered along the axial cell and by a single large nucleus generally occupying a central position. All adult Nematogens which show only one large nucleus in the axial cell are Nematogens by direct development, and, so far as I have been able to ascertain, never produce but one kind of embryo, namely, the vermiform. It may be convenient to designate such individuals primary Nematogens.

The Rhombogenic condition is introduced after a certain number of cells — seldom more than from four to eight — have arisen from the two primary germ-cells. Instead of pursuing further the primary, or Nematogenic mode of multiplication by simple division, each of these cells (»germogens« of Van Beneden) becomes a center of proliferation, producing cells which are compactly and more or less concentrically grouped around the mother cell, as described by Van Beneden. An interesting event takes place preparatory to the introduction of the Rhombogenic mode of cell-multiplication. This event, which completely escaped the attention of Van Beneden, consists in the elimination from the cell of a body analogous to a polar globule. Whether this body is the physiological equivalent of the polar globule so widly known among Metazoa, is a question I am unable to answer. The reasons to be urged against such an interpretation are important, though not quite conclusive to my mind. Nothing that could be called fecundation has yet been discovered; but the chief objection lies in the fact that the cell

which produces this corpuscle does not, apparently, develop directly to a Dicyemid, but produces germ-cells which so develop. I shall have occasion to return to this objection further on.

We may now trace the origin and fate of this corpuscle, and the history of the cell from which it arises. The earliest stage in its formation with which I have met is given in figure 104, Pl. 5. This figure represents apparently a nearly finished karyokinetic division, in which all, or nearly all, of the protoplasm remains with one of the daughter nuclei. The partially liberated portion (*n'*) is transparent, slightly granular, and shows a large nucleolus-like body in its external surface. The evidences of a karyokinetic division were more plainly indicated in the original drawing than the lithographer has represented. The plane of division was marked by what appeared to be short remnants of spindle-fibres, directed at right angles to it (incorrectly represented by mere dots in the figure). The fully liberated corpuscle, hemispherical, and still in contact by its flattened face with the producing cell, is seen in fig. 105. It is, in this instance, slightly larger than the central nucleus, and shows no distinctly defined membrane. It has all the appearance of being a nuclear body, and its subsequent history shows that it may be so regarded. It is not improbable that it contains a very small amount of cell protoplasm, and that from this is formed the thick membrane that arises soon after it becomes free. Be this as it may, it will later be impossible to distinguish this body from others of an undoubted nuclear character, and, as it remains for a considerable time near the proliferating cell, it may conveniently be called a paranucleus.

In fig. 106 (from a young *Dicyema typus*) the paranucleus (*n'*) lies detached by the side of two cells (*g*) which have arisen by division of the cell *m* in fig. 105. Fig. 107, from a *D. moschatum*, .5 mm long, represents a similar stage, in which the paranucleus already shows a double-contoured membrane. The multiplication of the two cells (*g* in figs. 106 and 107) by division soon leads to the formation of a cell-aggregate, such as is seen in figs. 108 and 109 *g*.

At this time one of the cells, generally occupying a central position, as seen in fig. 109, and in Van Beneden's figures 15 and 21, Pl. 1, is larger than the others, and incloses a nucleus (*n''*) that takes a sharper outline in acetic acid than is seen in the nuclei of the peripheral cells. This cell becomes the generatrix of successive generations of cells, all of which, if we except those of the last generation, are destined to produce infusoriform embryos in the manner described by Van

Beneden. This cell is evidently not identical with the cell *m*, figs. 104, 105, and the name germogen, originally applied to both cases, may therefore advantageously be restricted to the former; while the entire cell-group (*g*) may be called the Infusorigen, a term used by Van Beneden as a synonym for Rhombogen. A somewhat later condition is seen in fig. 110, taken from a young Rhombogen (*Dicyemennea Eledones*), measuring 1.15 mm. The figure shows everything that was present in the axial cell, namely, one Infusorigen, one paranucleus (*n'*), and one central nucleus (*ne*). The germogen presents, in addition to its nucleus (*n''*), six to eight germ-cells. These germ-cells have very delicate outlines, which become tolerably distinct after remaining a few moments in acetic acid; and they appear to lie within the generative cell, or germogen. The peripheral cells of the Infusorigen are more numerous than in stage 109, but they are still arranged in a single layer and form a hemispherical envelope around the germogen. The paranucleus has already attained a diameter considerably more than half that of the large central nucleus, and still lies near the place of its origin.

The still later form of the Infusorigen, seen in Fig. 111, differs in no essential point from the one just described. The axial cell, of which this figure shows only a part, contained one large central nucleus, lying midway between two Infusorigens. With each Infusorigen were associated mature germ-cells, cleavage-stages, young infusoriform embryos, and a single paranucleus. The posterior Infusorigen, the one seen in this figure, had five stages in front (only two given in the figure) and three behind, consisting of a germ-cell with fusiform nucleus (*ig*), an eight-cell stage, and an embryo (only outlined), and behind all the paranucleus (*n'*). The anterior Infusorigen had six stages in front and five behind, the paranucleus lying before all. In this young Rhombogen (2 mm long) we have then two proliferating centers (Infusorigens) and three large free nuclei (the central nucleus and two paranuclei). Thus we find that, where there is one active Infusorigen only, there are invariably two free nuclei, and no more; and where two such Infusorigens are present, the number of free nuclei is always three. As every Infusorigen is accompained by a paranucleus, and as the axial cell incloses a central nucleus at the outset, it is plain that in Rhombogens the number of free nuclei will always exceed the number of Infusorigens by at least one. Will the excess ever be more than one? The following observation answers this question. April 8 I found a Rhombogen (2 mm long) containing seven Infusorigens and nine free nuclei. Whence came the ninth nucleus? The further history of the Infusorigen will ex-

plain this point. Thus far we have seen only two kinds of free nuclei in the axial cell — paranuclei and the central nucleus. Another set of nuclei, quite distinct in origin and time of appearance, is now to be accounted for. Van Beneden has correctly stated the origin of this third class of nuclei, but he failed to recognize that their appearance marks the vanishing point of the Rhombogenic phase of life. »A germogen,« says Van Beneden, »can produce only a limited number of generations of germs. The cell gradually exhausts itself. The protoplasmic body of the cell is entirely used up in the formation of the last generation; the nucleus is then all that remains of the germogen. It is found at first in the middle of the rosette formed by the last germs grouped around it; but the rosette ends in disaggregation; the germs separate from one another, and the nucleus of the germogen is then found in suspension in the protoplasmic reticulum of the endodermic cell, which becomes polynucleated.« (1, p. 53—54.)

Thus there arise in the course of the history of every Infusorigen two nuclei: one, the paranucleus, appears as a preparatory step to the development of the Infusorigen; the other, which may be called the residual nucleus, is what remains after the role of the Infusorigen is played. As the several Infusorigens found in a single individual may not reach the concluding point of their productivity simultaneously, it follows that the number of free nuclei in a Rhombogen may exceed the number of Infusorigens by more than one; and this explains the case, above noted, of seven Infusorigens and nine free nuclei. One of the seven Infusorigens had ended its career, and perhaps we might say its existence. The paranucleus, the residual nucleus, and a loose group of cells were all there was left of it. Still it may be said that the number of free nuclei for the pure Rhombogenic state is one more than the number of Infusorigens, since the appearance of residual nuclei simply marks an epoch of transition from the Rhombogenic to the Nematogenic mode of reproduction. Allowing that such a transition takes place, and that the free nuclei arise in the manner described, we have an explanation of the plurality of nuclei in the axial cells of many Nematogens, and further, of the fact that the number of these nuclei is odd. A single nucleus in a Nematogen indicates that the individual is a Nematogen by direct development; three nuclei show that the individual has passed through a Rhombogenic stage in which only one Infusorigen was present; five, seven, or nine nuclei point in the same way to the prior existence of two, three or four Infusorigens.

Phenomena of Transition.

The closing events in the history of the Infusorigen form at once the end of one act and the beginning of another in the drama of reproduction. This transitional state is seen in figures 112 and 113, both taken from the same *Dicyemennea Eledones* (2.40 mm long). These two figures show all that the axial cell contained, except the central nucleus. We see here two paranuclei (*n'*), and the final products of two Infusorigens, namely, two defunct germogens (*c*), each inclosing a residual nucleus (*n''*), and two clusters of germ-cells (*vg*). The germogen has finished its work; its reproductive energy has departed. What becomes of its protoplasmic body I am unable to say; but it probably dissolves, leaving the residual nucleus free. The germ-cells are, in one case, loosely aggregated around the germogen, but they can no longer be said to form the envelope of an active Infusorigen. These germ-cells agree fully with the germ-cells found in Nematogens, both in size, in general aspect, and in their incoherent order. A comparison of these figures with 110 and 111 shows how widely different in these respects are the germ-cells destined to produce infusoriform embryos. The last germs produced by the germogen differ from those of preceding generations in being much smaller, in having relatively smaller nuclei, and in showing no tendency to form anything like an organic group. They differ still further in possessing the power of multiplication by division, as shown in figures 114, 115, where the loose arrangement of the cells corresponds exactly with what is seen in figs. 1 and 14, Pl. 1. In the Rhombogen, on the contrary, there is no multiplication by division outside the Infusorigen. The germ-cells (*ig*) in fig. 111 are undergoing developmental, not multiplicative division It is a process of cleavage rather than simple fission.

Fig. 117 contains two examples of a transitional kind. The *D. moschatum* from which this was taken contained, besides the central nucleus, four such assemblages as are seen in the figure, in all of which, except one, a residual nucleus was present. It is possible, if not probable, that this nucleus was present, although not visible, in the cluster *c*. The cells *vg*, seen in these figures, continue to multiply until, in many cases, the entire axial cell is loaded with them. Sooner or later they begin to develop into vermiform embryos.

The last generation of cells produced by the Infusorigen is not then homodynamous with those of prior origin. The appearance of this generation marks the introduction of a new order of propagation, —

it is the substitution of the Nematogenic for the Rhombogenic mode of reproduction. The individuals in which such a transition has already taken place may be called secondary Nematogens, and these may be easily distinguished from primary Nematogens by the occurrence of more than one large free nucleus in the axial cell.

Transitional Period. — The passage from one state to the other, in cases where the number of Infusorigens is more than one, may not begin simultaneously in these several centers; so that one portion of the individual may be occupied by one or more Infusorigens and various stages of infusoriform embryos, while another portion is filled with germ-cells destined to produce eventually vermiform embryos. Thus the two modes of cell-genesis may co-exist in the same individual; but this co-existence never, so far as I have seen, continues so long that the two kinds of embryos occur together. The infusoriform embryos desert the parent before the development of vermiform embryos begins.

The disappearance of the infusoriform embryos does not however bring the transitional period to a close. This period extends from the moment when, in any one of the Infusorigens, the production of infusorific germs ceases to the time when the proper vermific germ-cells appear, and the latter arise only after a considerable time has been consumed in the multiplication of cells by simple division. Essentially, it is a germ-producing period throughout, complicated only at the beginning by the co-existence of two modes of cell-genesis.

Simple as the history of this period is, it has absorbed more time than any other portion of my work. Evidences tending to establish the fact of a transition accumulated for a considerable time, before it became clear in what direction it takes place. If the dissemination of the species is provided for by the infusoriform embryos, it seemed natural, assuming that a change of generation takes place, to expect that these embryos would arise after the vermiform.

It seems from a citation before given, that this order of events was among the possibilities that occurred to Van Beneden, although he went no further than to simply state the question.

The co-existence of the two modes of generation in the same individual made it somewhat difficult to obtain a clear notion of the distinctive features of the Rhombogenic and Nematogenic conditions. The study of the development of the Rhombogen and the Nematogen, and a comparison of the two states in adult stages, gradually sharpened the contrast between them, and placed their essential characters in a clearer

light, thus preparing the way for discriminating between them even when intermingled. I have seen cases which, at the time of recording, seemed to me to favor the idea of a transition from the Nematogen to the Rhombogen; but I am now of the opinion that the transition is always in the opposite direction.

Transitional Phases. — The following cases are given as illustrations of what may be seen during the transitional period.

1. *Dicyemennea Eledones* from *Eledone moschata*, Mar. 31. Fig. 15, Pl. 1, represents a portion of a large individual, in which are seen the central nucleus (*ne*), two paranuclei (*n'*), two Infusorigens, and scattered cells (*vg*). No embryos nor embryonic stages. The two Infusorigens show all the parts of active Infusorigens, but their peripheral cells no longer exhibit the compact arrangement characteristic of earlier stages.

Largest peripheral cells .016 mm in diam., nuclei .01 mm—.012mm
Largest scattered cells .012mm - - nuclei .004 mm—.005mm

The difference in size between the nuclei of the two classes of cells is worthy of notice. The smallest cells of both kinds agree in every particular; the nuclei of the larger scattered cells differ but little from those of the smaller cells.

The paranuclei differ from the central nucleus only in size and form. Both present the thick membrane, the reticulum, and large vacuolated nucleoli. The germogen shown a residual nucleus, and, in one case, a rosette of six or more nuclei (?).

Not a single individual was found with infusoriform embryos, nor any approaching the pure Rhombogenic condition more nearly than the one here given. Many were seen in which the entire axial cell was filled with loose or scattered cells like those seen in the fig. (*vg*), and others differing from these only in having from one to many vermiform embryos.

I regard this as a Rhombogen already far advanced towards the Nematogenic state. The infusoriform embryos have all escaped, and the Infusorigens are in progress of breaking up into scattered cells.

2. *D. gracile*, from *Sepia officinalis*, Mar. 30. In two individuals vermiform embryos (two in each case) were found, and with them several Infusorigens accompanied by early stages of infusoriform embryos. Most individuals obtained from this Sepia were Rhombogens.

These two instances are the only ones I have ever met with, in which I could say with certainty that the two kinds of develop-

ment were progressing simultaneously in the same individual.

These individuals contained a central nucleus and as many paranuclei as Infusorigens. The few Nematogens present contained a very small number of embryos, generally not more than two or three. A considerable number of individuals containing only numerous scattered germ-cells were noticed.

It is not quite clear how these two cases are to be explained. It appears to me extremely improbable that the two modes of development were destined to be carried far enough to bring fully formed embryos of both sorts together, since no such instances have ever come to notice.

It is almost certain that one form of development was soon to be wholly superseded by the other. As I have not succeeded in finding any evidence that Nematogenic development is ever succeeded by Rhombogenic, I am led to suppose that the young infusoriform stages in these two Dicyemids were of an abortive nature, destined to disappear either by dissolution or by breaking up into separate cells. The only credible supposition remaining is, that the few already fully formed vermiform embryos were soon to escape, and that reproduction was henceforth to be purely Rhombogenic. This may be the correct view, but I have found nothing outside of these cases that appears to confirm it. In both of these Dicyemids a considerable number of loose germ-cells of the vermific class was found, and their presence is more easily reconciled with the former interpretation than with the latter.

3. *D. moschatum*, from *Eledone moschata*, Mar. 12. I shall here describe several individuals, and first of all two young Rhombogens and a young Nematogen, and then two cases of transition.

a. Rhombogen .45 mm long. The posterior two thirds of the axial cell contained nothing; in the anterior third were seen two large free nuclei (one .016 mm in diam., the other .020 mm), and between them a distinct Infusorigen and a single isolated germ-cell. The larger cells in the peripheral layer of the Infusorigen measured .014 mm, the average size of infusorific germ-cells.

b. Rhombogen 1 mm long. A little more than one half of the middle portion of the axial cell occupied by a central Infusorigen, preceded by a nucleus and seven stages of development, and followed by four infusorific germ-cells (.014 mm in diam.) and sixteen embryonic stages. Behind all came another free nucleus.

c. Nematogen 1.20 mm long. This was the only individual found that contained a vermiform embryo. The contents of the axial cell were

a single free nucleus, a single fully formed vermiform embryo, and numerous germ-cells measuring from .004 mm to .008 mm in diameter. The germ-cells were of the size and appearance of those seen in fig. 103, which undoubtedly represents a young Nematogen. The nuclei of the largest germ-cells were from .004 mm to .005 mm in diameter.

d. A Transitional 1 mm long. The first fourth of the axial cell occupied by an Infusorigen, six embryonic stages (three fully developed), and two nuclei; the remaining three fourths filled with scattered germ-cells .006 mm — .008 mm in diam., the larger with nuclei .004 mm in diam. Several of these germ-cells in process of division, others in which the division was just completed.

e. A Transitional .825 mm long. The posterior half of the axial cell contains nothing; the anterior half shows three free nuclei, one fully formed infusoriform embryo and three incompletely developed, and crowded germ-cells from .004 mm — .008 mm in diameter.

4. *D. moschatum* 1.80 mm long, from *E. moschata*, Feb. 7. A portion of this Dicyemid seen in fig. 40, Pl. 3. The axial cell contained besides three large free nuclei nothing but cells, which were crowded towards the two ends, but distributed in a loose row along the middle, as seen in the figure.

These cells were of two kinds, one spherical (.007 mm — .011 mm) with a large nucleus (.005 mm — .006 mm), and one ellipsoidal or fusiform (.007 mm × .015 mm) with smaller nucleus (.004 mm). The latter were often found in pairs, lying in close contact within vacuole-like spaces. In some cases the line of division between the two could not be clearly distinguished. In most of these elongated cells the protoplasm was striated in the direction of the longer axis.

From what I have seen in other similar cases, I believe these cells are in process of multiplication by division. It is probable that the spherical cells, after attaining a certain size, divide, producing two cells which assume the elongated form preparatory to a second division. This individual represents a transitional form derived from a Rhombogen in which there was but one Infusorigen.

5. *D. moschatum* 3.92 mm long, from *E. moschata*, Feb. 8. The anterior portion of the axial cell for about 1 mm occupied by numerous Infusoriform embryos emanating from a single Infusorigen. The larger infusorific germ-cells .016 in diam., nuclei .01 mm.

In the hind third of the axial cell a single free nucleus (.035 mm × .03 mm) with nucleolus .0025 mm, and numerous loose germ-cells

(.004 mm — .009 mm). Towards the anterior limit of this third the germ-cells became fewer and smaller.

The central portion of the axial cell, between the two end portions just described, was occupied only by three free nuclei, measuring (1) .05 mm × .04 mm, (2) .03 mm × .02 mm, and (3) .04 mm × .03 mm. Each nucleus contained a single nucleolus (from .0025 mm to .005 mm), except one which had two very small nucleoli.

One of the free nuclei was probably the paranucleus of the Infusorigen; one was the central nucleus (.05 mm × .04 mm); the remaining two were the paranucleus and residual nucleus of an Infusorigen that once occupied the portion now filled with loose germ-cells. The still active Infusorigen is destined to leave another residual nucleus, and a tract of loose germ-cells like that seen in the opposite end of the axial cell. Thus the time would come when the entire axial cell would be filled with germ-cells, and such cases are of frequent occurrence.

6. *D. moschatum*, from *Eledone moschata*, Feb. 12. In this Eledone, the size of which was not recorded, not a single individual was found containing vermiform embryos, although the renal organ was very thoroughly searched. Rhombogens and Transitionals were very abundant, and several colonies of extremely long individuals were found. Two cases may be described.

a. A Transitional 5.55 mm long, filled with an enormous number of germ-cells. No embryos nor embryonic stages, nor any trace of an Infusorigen. Only 6 nuclei were seen, but I think one must have been overlooked. These nuclei were located as follows:

(1) 2.30 mm from the head; size .05 mm × .03 mm; no nucleolus.
(2) 2.65 - - - - - .025 mm; 1 nucleolus.
(3) 2.80 - - - - - .03 mm × .02 mm; 1 nucleolus.
(4) 3.10 - - - - - .03 - × .02 mm; 2 nucleoli.
(5) 3.50 - - - - - .045 - × .03 mm; no nucleolus.
(6) 3.65 - - - - - .025 - × .02 mm; 1 nucleolus.

The nuclei were thus distributed along a central tract 1.35 mm long, or about one fourth the entire length. In the hind portion, extending forward to the 3^{d} nucleus, the germ-cells were densely packed; from the 3^{d} to the 2^{d} nucleus they became less numerous; and between the 2^{d} and 1st nucleus only five were found. The anterior portion for about 1 mm is again densely packed with germ-cells, and from this point backward to the 1st nucleus the number rapidly diminishes.

The germ-cells varied from .004 mm to .008 mm in diam.

This individual, judging from the contents of the axial cell, once

contained three Infusorigens, from two of which arose the longer, and from the other the shorter tract of cells. One free nucleus was probably concealed among the closely packed cells, and thus escaped notice.

b. A Transitional somewhat less advanced than *a*. The anterior portion was much stretched, and so the length could not be ascertained; but the posterior portion appeared not to have thus suffered.

At a distance of 1 mm from the hind end occurred a large free nucleus (.08 mm × .05 mm); in front of this, extending 1.20 mm, was an Infusorigen with its two series of germs in all stages of development. Next came a long tract of cells, extending through 3.90 mm, densely packed towards the middle; then a tract containing only two free nuclei; and finally, in the anterior end, another active Infusorigen with adjuncts similar to those seen in the posterior Infusorigen.

There were 7 free nuclei in this individual. The germ-cells of the central tract varied from .004 mm to .008 mm.

This specimen differs from *a* in that only one of the three Infusorigens has been replaced by loose germ-cells. Most of the smaller individuals were pure Rhombogens; but some of these also were in different stages of transition.

7. *D. moschatum* from *E. moschata*, April 13. This large Eledone contained many Rhombogens and Transitionals, but, so far as seen, no Nematogens with vermiform embryos.

One individual contained only numerous germ-cells from .004 mm to .008 mm in diam.; in another the germ-cells measured .006 mm to .01 mm, and many were in process of division. In nearly all cases where only numerous germ-cells were present, there were several free nuclei.

In one individual 2 mm long, I found two fully developed Infusoriform embryos, numerous germ-cells of the vermific order scattered from end to end, four free nuclei, and, near the hind end, a free germogen with its residual nucleus.

This Dicyemid had evidently passed a Rhombogenic stage represented by two Infusorigens.

As to the number of free nuclei, although I have never counted more than nine in any Nematogen, I think it probable that the number may in rare cases be as high as seventeen, as I have seen one Rhombogen with eight Infusorigens and nine free nuclei.

As cases of transition occur not only among the shorter individuals but also among the very longest, we may infer that the substitution of the Nematogenic for the Rhombogenic mode of reproduction does not

depend on the age of the Dicyemid. I am unable to assign any causes for such a change. Possibly it is in some way connected with the exhaustion of nuclear substance, or some element of this substance.

Germ-cells. — It has already been shown that vermific germ-cells differ, as a rule, conspicuously in size from infusorific germ-cells. In *Dicyema moschatum* I find the cells of the first class about two thirds as large as those of the second class. The average size of infusorific germ-cells, as given by Van Beneden, is .021 mm, while vermific germ-cells are said to vary between .012 mm and .014 mm. As the difference in size between these two kinds of cells furnishes one of the means of recognizing transitional phases, a few cases may here be given, supplementary to those already described.

Dicyema moschatum. — 1. Fig. 62, Pl. 5, represents one of the smallest (.0045 mm), and figs. 63 and 64 two of the larger germ-cells (.0085 mm) found in the same Nematogen. Both of the larger cells had attained their full size and were preparing to take the first step in cleavage, as indicated by the presence of spindle-fibres in the nucleus. These figures were drawn from acetic acid preparations, and care was taken to select such cases as could be said with considerable certainty to be entering upon a developmental, or cleavage division, rather than a multiplicative division.

2. Feb. 9. A small Eledone in which only Nematogens were found. In one individual containing a single embryo, the germ-cells ranged between .004 mm and .010 mm; in another (2.90 mm long) containing several embryos the numerous germ-cells varied between .004 mm and .008 mm. Both individuals were primary Nematogens, as shown by the single free nucleus.

3. Apr. 4. A secondary Nematogen, inclosing numerous embryos, and germ-cells varying between .003 mm and .008 mm. Osmic acid preparation.

4. Apr. 11. A long Nematogen inclosing in the posterior half embryos and germ-cells (.004 mm to .009 mm); in the anterior half numerous fusiform and otherwise elongated cells which were nearly all in process of multiplicative division. To find germ-cells in this condition, it is only necessary to take fresh Dicyemids and examine them while the acid is penetrating. Multiplication by division is certainly not a rare phenomenon although Van Beneden states that he never saw it.

Dicyemennea Eledones. — 1. Mar. 10. A primary Nematogen, 1.2 mm long, contained few embryos and germ-cells scattered along the

entire axial cell. Germ-cells (acetic acid) varied between .006 mm and .012 mm; the nuclei of the larger cells averaged .004 mm.

A second primary Nematogen, 1.60 mm long, inclosed no embryos; the germ-cells measured from .004 mm to .010 mm, the nuclei from .003 mm to .006 mm. The germ-cells were not numerous, and occupied only a little more than the middle third of the axial cell.

A third primary Nematogen, 3 mm long, inclosed three fully developed embryos and germ-cells varying between .006 mm and .015 mm with nuclei from .003 mm to .005 mm. Osmic acid.

A fourth primary Nematogen contained one embryo and germ-cells which varied from .006 mm to .014 mm. The larger cells were paler than the smaller ones, and a few inclosed two nuclei. Acetic acid.

A fifth primary Nematogen, 6.55 mm long, contained a few embryonic stages, and numerous germ-cells .006 mm — .014 mm in diam., with nuclei .003 mm — .005 mm in diam. Osmic acid.

A Rhombogen. 4 mm long, contained two Infusorigens with embryonic stages, and three free nuclei.

The mature germ-cells measured .015 mm and their nuclei .008 mm. Acetic acid.

2. Apr. 9. A primary Nematogen .75 mm long. The entire contents of the axial cell seen in fig. 101, Pl. 5. Here are two large round pale cells *vg* (.014 mm in diam.) and smaller cells that are much more coarsely granular. As the large pale cells are also found in Nematogens that contain embryos and numerous germ-cells, it seems to me probable that they are the mature germ-cells, and that they arise from the smaller and darker cells by simple growth.

There is not then much difference in size between the two kinds of germ-cells in this species. The nuclei of the infusorific germs are however here, as in all the species I have examined, conspicuously larger than those of the vermific class.

Dicyema truncatum Apr. 14. Fig. 99, Pl. 5, shows the contents of the axial cell of a young Nematogen .3 mm long. Besides the central nucleus (*ne*), there are five round germ-cells with small nuclei and a large fusiform cell with large nucleus. As I find in another individual (.45 mm long) only the central nucleus and two large fusiform germ-cells, I infer that the small germ-cells in fig. 99 have arisen by division of large fusiform cells.

The infusorigen. — Having traced the history of the Infusorigen from its origin to its dissolution, we may now consider briefly the question which this history raises in regard to its significance. Is it a

mere aggregation of indifferent cells completely destitute of all structural unity? or, does it show, for at least a time, a higher degree of coherency and a greater uniformity in the arrangement of parts than could be supposed to exist among cells having no structural relation with one another? If the second question be answered affirmatively, as I think it must, a third question at once arises concerning the degree of individuality exhibited by this body of cells. Is it the individuality of an organ, or of an independent organism? Although there is still much room for doubt on this point, there is certainly good ground for thinking that the Infusorigen represents an individuality of a personal order. Long before suspecting any analogy between the paranucleus and a polar globule, I was struck with the close resemblance of the Infusorigen to the Gastrula of the vermiform embryo. So nearly do they sometimes agree, that only the size and the presence of a paranucleus enable one to distinguish the one from the other. Before ascertaining the history of the paranucleus, and before therefore it could be used as a guide in this matter, I several times met with young Rhombogens whose Infusorigens were such perfect Gastrulae that they were at first mistaken for vermiform stages. Although the fate of the cells of the Infusorigen seemed at first to rob this resemblance of all significance, I am now disposed to admit not only that there is a possibility, but also even a probability that the Infusorigen and the vermiform embryo are co-ordinate forms. That this idea is not wholly fanciful becomes at once evident by comparing the Gastrulae seen in figs. 73, 74, 76 with the young Infusorigens in figs. 109 and 110. If any doubt arise as to whether my figures exaggerate the resemblance, it will probably disappear on consulting Van Beneden's figures 15, 20—24, Pl. 1, and 47 a, 48 b—56, Pl. 3. Fig. 109 represents a Gastrula in form and structure quite as perfectly as Fig. 73. It is an epibolic Gastrula in both cases, consisting of a large central cell partially enveloped by a single layer of smaller cells. Comparing the later stages, figs. 76 and 110, we see that cell-multiplication begins somewhat earlier in the germogen than in the axial cell: but all the essential Gastrula features are still preserved, and the germogen presents most striking analogies with the axial cell seen in the closed Gastrula of fig. 79.

I have never seen the nucleus (n'') of the germogen in process of division, and Van Beneden thinks that it never intervenes in the production of germ-cells. Before we can safely declare that it has nothing whatever to do with this production, we must be able to assign something better than a miraculous origin to the germ-cells. To assert that

they arrise de novo: is only a rather immodest way of saying that their origin remains to be determined. That there is some genetic relation between these cells and the nucleus of the germogen appears to me much more probable than any contradictory hypothesis. The points of analogy between the residual nucleus and the central nucleus of the vermiform embryo make it somewhat probable that the first cell, or cells that arise in the germogen are formed at the expense of both the nucleus and the protoplasm. The origin of subsequent generations of cells would then present no difficulty, even if the nucleus henceforth became inactive, as in the case of the nucleus of the axial cell. The passivity of the nucleus would only render the parallel between the germogen and the axial cell still more complete. Whatever be the role of this nucleus, the germogen agrees with the axial cell in being the generatrix of germ-cells, and in containing a nucleus that sooner or later becomes entirely inactive. The comparison is not at all impaired by the interesting difference, that the development of the germ-cells takes place in one case within, in the other outside, the parent cell.

The similarity between the germogen and the axial cell with respect to the formation of germ-cells called forth the following remark from Van Beneden: »Ces germes se forment donc dans les cellules germigènes à peu près comme les germes des embryons vermiformes dans le corps de la cellule endodermique.« I think this statement may be fully correct, although the opinion on which it was based, that the germ-cells in both cases arise, not from pre-existing cells, but as entirely new and independent formations, is certainly incorrect in one case and probably so in both.

It remains to consider to what extent the peripheral cells of the Infusorigen are comparable with the ectoderm of the vermiform embryo. That the peripheral cells pursue the path of ectodermal development for only a short distance and then abandon it altogether in becoming free germ-cells, is certainly a very interesting difference between the two layers; but this fact raises no insuperable objection to their morphological equivalence. It is not incompatible with our present knowledge of functional differentiation, to suppose that a layer of cells which has once played the role of an ectoderm, might, under conditions that render an ectoderm useless, revert to that kind of work to which all others are subservient, namely, reproduction. Such a reversion would not imply any fundamental change in the character of the cells; it would be simply a resumption of a function that had fallen into abeyance, as a result of those specializing conditions which bring about

a division of labor by exalting, in one group of cells, functions that are to a greater or less extent complementary to those exalted in other groups. A change of a similar nature occurs when an organism — *Sacculina* for instance, — removed from the struggle for existence by a parasitism that makes food and safty attainable without exertion, undergoes what is called retrogressive metamorphosis. The process of simplification brings the animal to a state in which the dissipation of force is minimized and the reproductive power enormously increased. It matters very little whether we call this process retrogressive or progressive, the result is evidently a great improvement in so far as the propagation of the species is concerned. The same result might be reached in a more direct way by arrest of development. In what other light can we regard Distomatous Sporocysts and Rediae?

It is doubtless exceptional that development should stop short with the Gastrula stage, and that every cell should share the reproductive power; but since the number of cells in the active enjoyment of this power may vary in different animals from one to many, it is certainly not incredible that it should include all, particularly if the organism, halting at a very early stage, begins to reproduce under circumstances of an extremely favorable kind.

When we reflect that in some animals (38, 50) the production of germ-cells is the peculiar work of the ectoderm; and further, that in the historical development of an animal not only may the same organ assume different functions at different times, but also the same function may shift from one set of cells to another (37), the fate of the peripheral cells of the Infusorigen ceases to be any serious objection to the view that they represent an ectoderm.

Although this view is admissible and apparently sustained by the figures of both Van Beneden and myself, the question of its tenability must be decided by the development of the Infusorigen. Van Beneden did not observe the earliest stages of this development, and hence very naturally inferred that the peripheral cells seen in his figs. 15, and 20—24, Pl. 1, and in my figs. 108—110, Pl. 5, arise within the germogen in the same manner as later generations of germ-cells. According to his statements, there is at the outset a large cell (germigène), within which there arise, independently of the nucleus, successive generations of cells that are disposed concentrically around the mother cell. This concentric arrangement is thus explained: »Quand les germes nés dans l'intérieur d'un germigène ont atteint un certain volume, ils se portent vers la surface; le protoplasme qui sépare les germes se contracte, il s'amasse

autour du noyau du germigène et par là les germes sont rejetés à la périphérie, puis éliminés. . . . . Dès qu'une première génération a été ainsi expulsée, il s'en forme un seconde: ces nouveaux germes naissent et se développent de la même manière que les premiers; ils sont expulsés à leur tour pour être remplacés par une troisième série, et ainsi les générations nouvelles refoulent peu à peu, de dedans en dehors, les générations plus anciennes. Il en résulte des couches concentriques de germes d'autant plus volumineux qu'ils sont plus loin du centre.« (1, p. 52—53.) Now all this applies to what comes after the stages represented in figs. 108 and 109. These stages are reached by repeated division of the cell *m* in figs. 104, 105; and this cell is not therefore identical with the central cell, or germogen seen in fig. 109 and later stages. The two-cell stage is seen in figs. 106 and 107, *g*. I have seen stages of three, five, six (fig. 108), and ten to twelve cells (fig. 109). I have not succeeded in tracing the origin of the germogen, i. e. I can not say precisely how early it appears. I have not been able to recognize it earlier than the six-cell stage, fig. 108; and in this case I could not clearly distinguish its boundary. In fig. 109, taken from the same individual, its outline is quite distinct and its nucleus very conspicuously different from the nuclei of the peripheral cells. It evidently arises quite early, and is one of the products of the division of the cell *m*. It is also certain that the peripheral cells seen in 108 and 109 continue to multiply by division as late as stage 110, and probably still later. By the time stage 109 is reached, or soon after, the germogen becomes the seat of endogenous cell-formation. How the first cells arise within the germogen, I have not been able to ascertain; but it is probable that they originate in the first instance by a division of the nucleus of the germogen, as is the case in the vermiform embryo. The successive generations might then arise in the same way, or, more probably, by division of the primary germ-cells.

Although my observations on the origin of the Infusorigen leave a number of important points undecided, so much is at least certain, that it does not arise from endogenously formed cells, but from a single cell, through a process of division analogous to, if not identical with, that of cleavage. I am unable to understand this fact on any other hypothesis than that the Infusorigen represents an individuality, of similar rank with the Gastrula of the vermiform embryo. Still I am not fully persuaded that this interpretation is correct. The important point which remains to be cleared up is the origin of the germ-cells within the germogen. If they arise in the same way that germ-cells arise in the axial

cell, the parallel between the Infusorigen and the vermiform embryo would be too complete to leave any room for doubt. No explanation can, however, be accepted, which fails to account for the fact that the history of the Infusorigen comprises two distinct periods; namely, a developmental period, in which a gastrula-like body is produced by a process comparable to cleavage, and a reproductive period, in which the germ-cells arise endogenously in a single central cell.

There is another point which must be noticed in this connection. How are we to explain the cohesion of the peripheral cells on any supposition that ignores or denies the individuality of the Infusorigen? If these cells have no organic relation with one another, why do they adhere to the germogen and to each other by plane faces? If there is no bond of union between them more than exists among independent germ-cells, why does there invariably arise a group, like that seen in figs. 109 and 110? Nothing of all this is seen among the germ-cells of the Nematogen. So long as germ-cells multiply by simple division, they show no disposition to arrange themselves into regular groups: the products of division at once separate, and if they come into contact with others, their independence is still attested by their spherical form. The whole axial cell may be crowded with such cells, and nowhere any semblance of an organic assemblage. The cells are like so many marbles confined within a certain area, in free rolling contact. As soon as developmental division begins in any one of these cells, a group is formed, which eventually becomes a vermiform embryo. We have seen also that the Rhombogenic stage is preceded by one in which the germ-cells maintain the freedom characteristic of vermific germ-cells. As soon as one of these cells has eliminated the nucleus-like corpuscle which we have called paranucleus, a kind of developmental division begins and a group is formed to which we have applied the name Infusorigen.

If the Infusorigen arose by endogenous cell-formation, the cohesion of its peripheral cells for a short time would require no further explanation than a mere description of their mode of origin. Their adhesion to each other and to the germogen would be simply the result of mechanical pressure, which further growth would overcome, and thus lead to complete separation. Such, presumably, was the view taken by Van Beneden, since no comments were added to the following statements:

»Ils [the peripheral-cells] ne se détachent que quand ils ont atteint leur complet dévelopement. Tant qu'ils adhèrent au germigène, la surface de contact est plane: les germes ont tous la forme d'une sphère tronquée et le germigène est limité par des faces planes se coupant

sous des angles dièdres; les germigène a une forme polyédrique.« (1, p. 53.)

Since the peripheral cells do not — at least in the first instance — arise endogenously, but by division, their cohesion evidently requires an explanation.

If the view here presented be the correct one, an interesting question arises, to which I am unable to give any positive answer. Does the first generation of cells produced by the germogen displace the peripheral cells previously formed by division? In other words, is the peripheral layer of the Infusorigen, in stages later than fig. 110, a product of the germogen? and is this layer replaced as often as a new generation of cells arises within the germogen?

The description given by Van Beneden would answer these questions in the affirmative. I have tried in vain to satisfy myself on this point, but I may remark that such an answer would lead us to suppose that a considerable number of Infusorific germ-cells would come to maturity simultaneously. Fig. 111 represents a typical case, from which I have never seen any very wide departures in young Infusorigens. In this instance, not more than two germ-cells can be said to have reached maturity at the same time. The fact that the stages of development are more and more advanced as they recede in either direction from the Infusorigen, shows plainly that not all of the peripheral cells can arrive at maturity at the same time. So far as I can learn, the germ-cells leave the Infusorigen at maturity, or shortly before, generally one or two at a time. In size and general appearance the ripe germ-cells agree with the larger cells of the peripheral layer, and I see no reason to doubt that they arise directly from this layer. If this be the case, this layer is probably replaced gradually by cells originating in the germogen. The comparison of the Infusorigen in its earlier phases with the developmental stages of the vermiform embryo is not, however, affected by the answer which may be given to these questions.

A Review of the Subject of Reproduction.

The phenomena of reproduction in the Dicyemids are in some respects unique, but, for the most part, not without analogies. It is my object to present here a general survey of these phenomena so far as now known, to interpret them so far as I may be able, and to consider some of their bearings.

We do not yet know how the Dicyemids pass from one Cephalo-

pod to another, and hence it remains doubtful whether all, or even the chief events of reproduction take place within the renal organ. Viewed comprehensively, and without reference to detail, what we actually know about this matter may be briefly summed up as follows:

1. Two kinds of embryos, differing very widely from each other in structure, form, size, and development, are set free within the renal organ of the Cephalopod.

2. Both sorts of embryos originate in a single parental form, and not in two unlike individuals as hitherto supposed.

3. The vermiform embryo develops directly into the adult; the fate of the infusoriform embryo remains still a matter of pure conjecture.

4. Although all adult Dicyemids of the same species are alike in structure, and essentially so in form, size, etc., they do not all take a like share in the work of reproduction. Some produce only one kind of embryo, and may therefore be called monogenic; others produce, successively, both sorts of embryos, and on this account may be named diphygenic[1].

5. Both monogenic and diphygenic individuals develop from the same kind of germ-cells, and no distinction between them arises until they begin to reproduce.

6. In monogenic individuals, the germ-cells, after a period of multiplicative division, develop into vermiform embryos.

7. In diphygenic individuals, after a much shorter period of multiplicative division, each germ-cell eliminates a nucleus-like corpuscle, and then develops into the Infusorigen, a group of cells comparable to the Gastrula of the vermiform embryo. From one to eight Infusorigens are found in one Dicyemid. The Infusorigen, after producing a certain number of germ-cells that give rise to infusoriform embryos, resolves itself into cells destined to produce vermiform embryos. The development of both kinds of embryos is completed within the individual which produced the Infusorigen.

The Infusoriform Embryo. — As vermiform embryos develop directly into adult Dicyemids, and as the multiplication of individuals within the host seems to be thus fully provided for, it has been generally assumed that the infusoriform embryo, which is not known to fulfill any co-operative purpose, serves the important end of carrying the species from host to host.

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[1] διφυής, of a two-fold nature, or form, and γεννᾶν, to produce.

Kölliker (4, p. 65) has suggested that this strange embryo may stand in the same relation to an unknown sexual form as *Cercariae* to sexual *Distoma*, and that the Dicyemids are only asexual generations standing between the hypothetical sexual individuals and the infusoriform embryo.

Van Beneden's earlier researches on the Dicyemids led him to the following conclusions: — »J'ai dit plus haut que les *Dicyema* s'altèrent, se désagrégent et périssent dans l'eau de mer. Ceci est vrai non-seulement pour les adultes, mais aussi pour les embryons vermiformes. Quant aux embryons infusoriformes, j'en ai conservé parfaitement vivants dans un verre de montre pendant deux, trois, quatre et même cinq jours, sans qu'ils aient subi, après ce séjour dans l'eau de mer, la moindre altération. Comme je n'ai jamais trouvé dans les reins des Céphalopodes aucune forme de transition entre un embryon infusoriforme et un *Dicyema*, j'en conclus que les *Dicyema* ne se multiplient, dans les corps spongieux du Céphalopode infesté, que par les embryons vermiformes. Mais comment l'espèce se transporte-t-elle d'un Céphalopode à un autre? Puisque les embryons vermiformes ne peuvent vivre dans l'eau de mer, il est clair qu'ils [ne] peuvent servir à propager l'infection parasitaire d'un Céphalopode à un autre. Ce ne peut être que par les infusoriformes que cette transmission s'opère. Je pense donc que les embryons infusoriformes quittent les corps spongieux des Céphalopodes chez lesquels ils sont nés; qu'ils vont à la recherche de jeunes Céphalopodes non encore infestés par les parasites et qu'ils servent ainsi à propager l'espèce d'un individu à un autre. Le passage se fait-il directement ou par l'intermédiaire d'un hôte dans lequel l'embryon infusoriforme accomplirait une partie de son évolution? Est-ce l'embryon lui-même qui se transforme en un jeune *Dicyema* ou bien est-ce le contenu cilié de l'urne qui constitue le germe destiné à reproduire l'espèce? L'embryon infusoriforme se modifie-t-il à la longue dans l'eau de mer et subit-il dans ce milieu des transformations avant d'arriver à l'individu auquel il doit donner le parasite? Ce sont là autant de questions auxquelles je ne puis répondre.« (1, p. 67—68.)

I have made several trials to ascertain how long these embryos would live in sea-water, sometimes keeping them in a watch-glass in a damp chamber; at other times in a glass tube in which the water was supplied with air by an aërating apparatus. A few drops of the renal fluid containing the embryos together with a few Dicyemids were placed in the tube, or watch-glass, and then sea-water added. The infusoriform embryos always swam about very lively on the addition of the

water, the larger fore end of the embryo describing a spiral while rotating. In no case have I succeeded in keeping them alive 48 hours. At the end of 24 hours many are usually found dead, or nearly so, the rest surviving only a few hours. The refractive bodies remain apparently unchanged for some time after death and after the disintegration of the other parts. I have not seen that the Dicyemids die sooner than the infusoriform embryos.

If the renal fluid contains a large admixture of sea-water, as maintained by VIGELIUS (52), it is not at all surprising that both embryos and adults live for a considerable time in nearly pure sea-water. But if the infusoriform embryos are able to live much longer than the vermiform embryos and the adults, as VAN BENEDEN's experiments seem to show, this fact alone would establish a very strong probability in favor of the opinion that they serve to carry the species to new hosts. It is possible, however, that the vermiform embryos fulfill this office, in which case the infusoriform embryos could only be interpreted as males. This alternative view is the one now entertained by VAN BENEDEN (2). There can be little doubt that one or the other of these views is correct; but the probabilities are so evenly balanced that it is difficult to become a partisan of either. The researches of GIARD (14—16), METSCHNIKOFF (17—19), and JULIN (20—21) make it clear that the *Orthonectidae* are closely related to the Dicyemids; but the parallel between them with respect to the phenomena of reproduction is not so complete as represented by VAN BENEDEN and JULIN, even if we may assume that the infusoriform embryo is a male. The following summary of conclusions arrived at by JULIN, will show some weak points in the argument from analogy.

1. There are three distinct kinds of adult individuals, one male and two female. Both female forms arise from the eggs of the so-called flat female (»forme aplatie«), the males alone being produced by the cylindrical female (»forme cylindrique«).

2. The distinctions between the females are slight compared with those between the two sexes. Their origin and earlier stages of development are the same, and it is only at a comparatively late date that it becomes possible to distinguish them, by a peculiarity of unknown significance, which appears only in the flat female. This peculiarity arises in the form of a cell-like body located near the anterior end, between the ectoderm and the cell-layer which later becomes the muscular layer. METSCHNIKOFF suggests that this body (»sub-polar cells«) may represent a rudimentary digestive tube.

3. Both female forms are oviparous, and produce eggs that are alike in size and general characters: but the mature eggs of the cylindrical female are expelled from the mother, and are completely independent of one another; while those of the flat female are never expelled, but remain united by a granular substance, in small masses that are nothing but fragments of the mother.

4. In both sexes the whole central cell (»globe endodermique primitif«) breaks up, before the close of the blastopore, into cells from which arise later the muscular layer and the numerous germ-cells.

5. Both of the adult female forms are supposed to be able to escape from the host in which they have developed and matured, and to swim free until they meet with a new host into which they may penetrate.

6. Arrived in the new host, the cylindrical female ends its existence in the act of expelling its eggs; the flat female ends by breaking up into fragments which become so many sacs (»Plasmodiumschläuche«, Metschnikoff) each inclosing a number of ova.

7. It is probable that the eggs of the cylindrical female are fecundated at the time of expulsion, »les spermatozoides pouvant être amenés par l'eau«; but it is left entirely undecided whether the eggs of the flat female are fecundated, or develop parthenogenctically.

As to the Dicyemids, nothing in the nature of fecundation has yet been discovered, and presumably nothing of the kind occurs in the monogenic individuals. In the case of the diphygenic individuals, there is perhaps some reason to suspect that fecundation introduces one of the two modes of reproduction. The chief ground for the suspicion is, however, the absence of any other equally plausible explanation of the origin of those peculiarities which distinguish Rhombogenic reproduction.

In his earlier paper, Van Beneden says, — »J'ignore également si la reproduction des Dicyémides se fait exclusivement par voie agame ou si la production des embryons de l'une ou de l'autre forme est précédée d'une fécondation véritable J'ai observé quelques faits qui me font pencher vers cette dernière alternative. Peut-être la production des Infusoriformes est-elle précédée de la fusion d'une cellule ectodermique avec la cellule endodermique de l'embryon vermiforme.« (1, p. 69.) What these facts are is not stated. Perhaps the supposed variability in the number of the ectodermal cells in the diphygenic individuals was at the bottom of this conjecture.

In this connection an observation may be mentioned, to which I should not hesitate to attribute considerable importance, had it been

frequently made. I have met with only four instances. April 8, I found in a large *D. moschatum*, containing vermiform embryos, an enigmatic ciliated body, resembling in shape and size an Infusoriform. I could distinguish the outlines of only a few cells. April 9 I obtained another individual of the same kind (4mm long), in which, just behind the single free nucleus, a similar body was found. In this case I could distinguish plainly six cells, and the resemblance to an Infusoriform was so close that I no longer hesitated to regard it as such. Again on April 12, I found two of these bodies within the same Nematogen; and under the same cover was another individual in which there was only one such body. In all these cases the bodies were undoubtedly modified forms of Infusoriforms. These four Dicyemids were all obtained from small Eledones, in which most of the parasites were Nematogenic. I was unable, except in one case, to determine whether the axial cell contained more than one free nucleus. The Cephalopods were killed by cutting off the head, and the examinations made immediately, so that I am perfectly certain that I had fresh Dicyemids. I am also equally certain that these Infusoriforms did not originate in the individuals in which they were found, but had penetrated from without, and were fast undergoing alterations, the precise nature of which I could not ascertain. It is possible that such occurrences are not so rare as they now appear to be; and that they have been overlooked either because they are seasonal, or confined to young Cephalopods. If the Infusoriforms be males, then a very probable explanation could be found for these occurrences, and, further, for the fact before noticed, that Nematogenic individuals prevail in young Cephalopods. The species would be carried to new hosts by the vermiform embryos; and after a certain number of parthenogenetic generations, or simultaneously with the females, would arrive the males. The males would penetrate the females, the ova be fecundated, and a generation of vermiform embryos arise, which would develop into diphygenic individuals. This is, to be sure, all conjecture; but it is nevertheless one of the possibilities which it may be advantageous to keep in mind in future investigation of this matter. If, however, there is no fecundation, or if it precede the elimination of the paranucleus, then it would be necessary to regard the five Infusoriforms found in four Nematogenic individuals, as stragglers.

The Infusorigen. — If the Infusorigen represents an individual, as I am strongly persuaded, then it must be regarded not only as a form brought about by arrest of development, but also as one whose personality and independence have been largely sacrificed in the

interests of a precocious and all-absorbing reproductive activity. In failing to reach the ancestral condition, it failed also to reach a free and independent existence. It lives, reproduces, and expires within the mother, and its two kinds of offspring are brought to complete development under the protection, if not at the expense, of this same mother, which thus sustains the relations of a host not only to its own children, but also to its more numerous grand-children. Remarkable as such relations certainly are, they present nothing fundamentally new to the helminthologist. The Infusorigen — according to this view — represents an individual which never gets beyond an embryonic condition, but which nevertheless reproduces agamogenetically while enjoying the protection of the parent, the latter holding to the Infusorigen relations similar to those of a Distomatous sporocyst to its Cercarian offspring, or to an intermediate generation of Radiae or sporocysts. That the protection of the parent should extend to the third generation is a phenomenon with which we have been familiar since the investigations of Von Siebold (46) and G. Wagener (49, p. 768) on *Gyrodactylus*. According to both authorities the fourth generation arises before the birth of the second; and G. Wagener states that even the fifth generation is already in progress of development by the time the second generation (Tochter) is born. Van Beneden (1, p. 48) says that he has seen a vermiform embryo within a vermiform embryo before the latter escapes from the parent. Carus mentions an instance of a sporocyst (Amme) containing Cercarian embryos within a larger sporocyst (25, p. 12). The only difference between such cases and the one here considered is, that in the former the successive generations are included the one within the other, and set free in the order of their origin; in the latter the second generation (the Infusorigen) is never set free, and consequently the third generation is thrown directly under the protection of the first. The existence of the Infusorigen has been abbreviated to such an extent that its maternity, so to speak, is practically obliterated, or replaced by that of its own parent. It is hardly necessary to remark that we see here the possibility of a complete extinction of an intermediate generation. The nature and general bearings of such phenomena have been thoroughly and comprehensively discussed by Leuckart (44, p. 118—153, and 43, p. 81—119).

The production of two kinds of germ-cells by the Infusorigen is an interesting, though not an isolated fact. It is well known that many rhabdocoelous *Turbellaria* produce »summer« and »winter eggs«; and, according to Metschnikoff (19, p. 299—300) and Korschelt (39,

p. 399), there are two distinct kinds of ova in *Dinophilus*, the smaller of which develop into the extremely small males, the larger, into females. Precisely the same sexual differentiation occurs in the summer eggs of Rotifers. Whether the difference betwen the two kinds of germ-cells of the Infusorigen is one of sex or of generation, is a question which I cannot answer. The comparison before introduced between the Dicyemids and the Orthonectidae does not reveal a resemblance sufficiently close to serve as a guide in this matter. After the discovery of two females in the Orthonectidae by JULIN, the parallelism between the evolutionary cycles of these parasites and the Dicyemids appeared to be complete, provided the Infusoriform could be regarded as a male. So complete seemed the analogy in other respects, that VAN BENEDEN did not hesitate to adopt this view of the Infusoriform and to incorporate it in the table of classification appended to his last paper (2).

While I see no insuperable objection to such an interpretation, and fully concede that the history of the contents of the urn, so far as known, renders it plausible, still decisive evidence is wanting, and the argument from analogy is far from being satisfactory.

In neither of the two female forms described by JULIN do we find anything comparable with the Infusorigen, nor anything at all resembling the transitional phenomena before stated. The »cylindrical female« produces males, and can not therefore be said to correspond to monogenic Dicyemids which produce females, nor to diphygenic Dicyemids which produce Infusorigens, or, — passing the Infusorigens — females and Infusoriforms. The »flat female« produces a dimorphous female progeny, and cannot therefore be compared with diphygenic Dicyemids, unless the Infusoriforms are females; nor with monogenic Dicyemids, unless the offspring of the latter be of two kinds. Some of the offspring become monogenic, others diphygenic; but it is by no means certain that this distinction originates in a difference of individuals. There is nothing in their origin and development, nor in their adult form or structure, which authorizes the opinion that they are unlike. Still it must be admitted that monogenic and diphygenic individuals may be primarily distinct, since we do not yet know what determines the germ-cells in one case to develop into vermiform embryos, and in the other, into Infusorigens. However this may be, the parallelism between the »cycle evolutif« of the Orthonectidae and that of the Dicyemids is not sufficiently complete to enable us to decide on the nature of the Infusoriform.

Chapter III.

The Development of the Vermiform Embryo and the Origin of the Germ-cells.

With respect to the cleavage and the formation of the epibolic Gastrula, my observations differ but little from those of Van Beneden; but I am unable to confirm his statements on the origin of the germ-cells and on the relation of the »oral pole« to the blastopore.

The first cleavage of the germ-cell always, so far as I can judge, results in two unequal parts, as seen in fig. 61 (*D. microcephalum*) and fig. 65, Pl. 5 (*D. moschatum*). The same is true of the Orthonectidae according to Julin. It often happens that one finds germ-cells divided into two equal parts, but I believe these are all cases of multiplicative division, not of cleavage. The cleavage is introduced by the formation of a karyolytic figure (fig. 63), in which I have been unable to recognize polar asters. I have reason to believe, however, that these asters are present even where they seem to be wholly wanting[1]. The spindle-fibres are found in the central as well as the peripheral portions of the nuclear area (See optical section, fig. 64), similarly as shown by Mark in the egg of Limax (45, p. 229). The two cells resulting from the first cleavage increase considerably in volume before the second cleavage, and they do not divide simultaneously. I am not able to say with certainty which cell divides first, but probably it is the smaller. The three-cell stage is followed by a four-cell stage, in which the two pairs of cells lie et right angles (figs. 69 et 70). One of the four cells — the larger — soon takes the position seen in fig. 71, the other three cells forming a sort of cap. At this stage the smallest of the four cells is quite as large as the original germ-cell. From the interesting fact that the volume increases as the cleavage advances[2], we may infer that considerable intervals elapse between successive cleavages. The large cell takes no further part in the cleavage, but becomes gradually enveloped as the number of cells in the ectodermal cap increases.

An optical section of the eleven-cell stage (fig. 74) shows the large central cell inclosed in an ectodermal mantle, which is open only on one side. Figs. 76 and 77 represent an optical section and a surface view of

[1] In the germ-band period of Clepsine, I find many small cells in which the karyolytic figures, seen from one side, show only the spindle; in others, where the figurs are cut obliquely, the asters are distinctly visible.

[2] Julin has observed the same in the Orthonectidae.

the Gastrula just before the close of the blastopore. I find in most cases that the ectodermal cells multiply more rapidly on one side of the blastopore than elsewhere (fig. 78), and this leads to the asymmetrical pyriform shape seen in figs. 80, 81 and 87. As the blastopore closes, the embryo lengthens and becomes more or less obtusely pointed at the blastoporal end. The broader end corresponds to the cephalic pole of the adult, and the pointed end to the opposite pole.

Van Beneden states that the anterior pole (»pôle oral«) corresponds to the blastopore (1, p. 48), and Julin maintains the same for the Orthonectidae. On this point Julin's statements are self-contradictory, as the following citations plainly show: — »A ce moment (fig. 15 et 16), l'ectoderme est constitué par huit cellules disposées en deux rangées: la première rangée, constituant l'extremité antérieure de l'embryon futur, est formée par deux cellules: l'autre rangée est représentée par six cellules.« (21, p. 24.) In a footnote of the succeeding page occurs the following remark: »Bien qu'il soit impossible de décider d'une façon positive si le blastopore chez l'embryon de *Rh. Giardii* est situé au pôle antérieur ou au pôle postérieur, ainsi que le démontrera la suite du développement, cependant, par analogie avec ce qui existe chez la femelle, où bien manifestement le blastopore est situé à l'extremité antérieure de l'embryon, nous admettons qu'il doit en être de même chez le mâle.« With reference to the female forms, Julin thinks that his figures 6, 7, 9, 10, 11, 12 and 13, Pl. 3, lead to the conclusion that »c'est à l'extremité antérieure de l'embryon que les cellules ectodermiques se rejoignent pour constituer une couche complète autour de l'endoderme. Le blastopore est donc situé chez la femelle à l'extremité antérieure du corps« p. 30. I confess that I am unable to see how the figures referred to establish the conclusion. The two caudal cells are seen in Dicyemids as early as the stage represented in fig. 88; and it is about this time, or a little later, that the whole number of ectodermal cells are present. With few exceptions (fig. 90) the anterior end of the embryo maintains a width equal to that of any portion of the body, as seen in figs. 91 and 92. The hind end, on the contrary, preserves a somewhat pointed form. The fully formed embryo (fig. 1, Pl. 1) still shows the same backward taper seen in fig. 91. The axial cell lies at first in the fore part of the embryo (figs. 86, 88, 89), the caudal cells forming a solid mass; but it gradually elongates into the caudal portion. Cases like fig. 90, led me at first to think that the polar cells develop from the pointed end of the embryo; but a careful examination of many embryos between the stages

represented in fig. 78 and fig. 91, has fully satisfied me that the broad end of the pyriform embryo (upper end in the figures) corresponds to the cephalic pole of the adult; in other words, that the main axis of the Gastrula coincides, with that of the complete animal. If JULIN's conclusion be correct, then we have to admit a remarkable difference in two cycles of development otherwise closely similar.

The Origin of the Germ-cells. — The first germ-cell appears after the close of the Gastrula, about the time the pyriform stage is reached. The nucleus, of the central cell undergoes a karyolytic division (figs. 8 and 85), the plane of division being at right angles to the axis of the embryo. Two nuclei are thus formed, the larger of which takes nearly a central position in the mother cell, the smaller lies near the hind end of this cell. I can not say whether during the division of the nucleus a portion of the protoplasm of the central cell is actually split off, or whether the protoplasmic body of the germ-cell forms endogenously. I have seen no case in which the nucleus of the germ-cell was free, and it seems probable that immediately after its formation an area of protoplasm becomes delimited around it. The body of the germ-cell differs in its appearance from the protoplasm of the central cell, being less granular and somewhat darker[1]. The second of the primary germ-cells makes its appearance at the fore end of the central cell, or, as we may now call it, the axial cell (fig. 89). I have seen but one case in which the second germ-cell lay behind the central nucleus. Although I have not succeeded in tracing the origin of this cell to the nucleus of the central cell, I think there can be little doubt that it arises in precisely the same manner as the first. The central nucleus takes no further share in the formation of germ-cells. From these two primary germ-cells arise all the germ-cells that appear later in the axial cell. Sometimes before, but generally soon after birth, multiplicative division begins, each of the primary germ-cells dividing into two (fig. 95). Frequently, perhaps always, the posterior cell divides first (fig. 94). After each division the resulting cells consume some time in growth before again dividing. In fig. 96, the posterior pair of small cells have arisen by the division of a cell like the large cell immediately in front of them. In fig. 97, two such pairs of cells are seen. Thus there is a slow multiplication of germ-cells going on during the growth of the embryo into the mature form.

[1] These appearances remind me of the formation of germ-cells in Triton, as described by Mr. IWAKAWA of Tokio (36, p. 267).

In the male Orthonectidae, a single cell is formed at each pole of the central cell, and these cells multiply subsequently by division, and eventually produce a layer of muscle-fibres that envelop the central cell, or its products. Although these cells do not serve as germ-cells, they are ontogenetically comparable with the primary germ-cells of the Dicyemids.

So far then as concerns the germ-cells of the vermiform embryo, their origin must be referred to the central or axial cell, the nucleus of which participates only in the formation of the first two. There is every reason to believe that the infusorific germ-cells have also a genetic relation with the nucleus of the germogen, although this point has not yet been established.

The account given by Van Beneden with respect to the origin of the vermific germ-cells is as follows: — »Les germes des embryons vermiformes se forment par voie endogène. Ils naissent isolément, souvent loin de tout germe préexistant, dans le reticulum de la cellule axiale. Ils apparaissent sous forme de petits corps sphériques à contour bien défini. Ils sont d'abord homogènes et ils présentent à leur centre un petit globule punctiforme. Le contour devient progressivement plus épais: bientôt on distingue autour d'un noyau plus clair, nucléolé à son centre, une zone peu épaisse d'une substance plus foncée. Cette zone s'epaissit progressivement et devient le corps de la cellule-germe.« (1, p. 41.) Again on page 43 he says, »Je crois pouvoir affirmer que tous les germes, chez les Nématogènes, se forment de cette manière. Jamais on ne trouve de germes en voie de division, si ce n'est ceux qui, arrivés à leur complet développement, se fractionnent pour donner naissance à un embryon.« As before pointed out, Van Beneden maintains the same mode of origin for the infusorific germ-cells.

No observations, were made in regard to the formation of germ-cells in the Heterocyemids (2, p. 203).

Endogenous Cell-formation. — The terms »free« and »endogenous« have each been used in two very different senses: they have been applied to nuclei and cells which arise within other cells, either (1) by division of the nuclei of the parent cells, or (2) independently of pre-existing nuclei, as formations de novo. To avoid ambiguity, it will be well to restrict the use of the word endogenous to cases of the first kind, and to employ, for the second, the word neoplastic.

It is a well established fact that cells arise endogenously in a very large number of cases among both plants and animals. The literature on this point has, in part, been noticed elsewhere (51). On the other-hand, the cases in which nuclei, or cells, have been affirmed to have a neoplastic origin (»autoplastic«, Lankester, 42) are much less numer-

ous, though not rare. One thing is observable in respect to these assumed neoplastic formations, that their number is constantly diminishing. It has happened over and over again that nuclei claimed to be neoplastic, have turned out, on re-examination with better methods, or with greater care, to be nothing more than ordinary endogenous nuclei. This has occurred so frequently, that it would now not be very rash to predict that another decade will completely exhaust the list. As to the cases still remaining, it is very clear that the best evidence for them is the want of evidence against them. Strasburger (47, 48), who among botanists, has given most attention to this subject, and who in his earlier writings maintained neoplastic nuclei to be of very general occurrence, now thinks that nothing of the kind occurs in the vegetable kingdom. Mark, who has recently reviewed this question, considers it »extremely doubtful whether new nuclei arise in animal cells without the least visible connection with the nuclear substance of preexisting nuclei.« (45, p. 545.)

Where the contrary has been asserted, as in the regeneration of epithelium (28), *Gregarina gigantea* (23), ciliated spores of *Acineta divisa* (30)[1], infusorific germ-cells of Dicyemids, etc., the negative results must be attributed either to over-hasty examination, or to exceptional difficulties in the way of obtaining reliable information.

Chapter IV.

Systematic Affinities.

Krohn and Erdl expressed no definite opinion on the systematic relations of the Dicyemids. In a review of the helminthological productions of 1843 and 1844 (12), Siebold records his opinion in the following words.

»Ref. möchte diese sonderbaren Wesen mit ihrem infusorienartigen Inhalte für die schlauchartigen Larven eines dem Generationswechsel unterworfenen Thieres halten. Derselbe kann dabei nicht umhin, auf die Ähnlichkeit dieses infusorienartigen Inhalts mit den räthselhaften, von Joh. Müller[2] beschriebenen ungeschwänzten Psorospermen aufmerksam zu machen.« p. 247.

The production of two kinds of embryos by Dicyema led Kölliker

[1] R. Hertwig (33) states that the nuclei of the spores are derivative.

[2] »Über eine eigenthümliche krankhafte parasitische Bildung mit specifisch organisirten Samenkörperchen.« Müll. Arch. 1841, p. 477.

(4) to regard them, not as completely developed animals, but as larval forms, comparable with the sporocysts of Trematodes. »Gestützt auf Steenstrup's Beobachtungen, dass bei den Distomen aus dem ursprünglichen Embryo ein erster Keimschlauch hervorgeht, in diesem eine Menge ganz gleicher Keimschläuche der zweiten Generation sich bilden und erst in diesen in ihrer Form abweichende Thiere, Cercarien, entstehen, die endlich in wahre Distomen sich umwandeln, ließe sich annehmen, dass die Mutterthiere von *Dicyema* mit wurmartigen Embryonen, welche letztere wieder zu ähnlichen Mutterthieren werden, Keimschläuche der ersten Generation oder Großammen sind, die wurmartigen Embryonen dagegen und die Mutterthiere mit infusorienartigen Embryonen den Ammen entsprechen, und endlich die infusorienartigen Embryonen mit den Cercarien auf einer Linie stehen.« p. 64—65. Three facts are mentioned as opposed to this view: namely, (1) it has not been shown that the Dicyemids, arise from true ova; (2) the infusoriform embryos have not the least resemblance to Cercariae: and (3) there are no indications of a transformation of the infusoriform embryo into a sexual animal. Still Kölliker holds it highly probable »dass *Dicyema* nur ein jugendlicher Zustand eines anderen Thieres, sei es nun eines Entozoon oder vielleicht einer Planarie, Nemertine, u. s. w. ist«.

»Bei jedem Dicyema,« says Wagener (5, p. 363), »bemerkt man zu beiden Seiten des Leibes zwei helle durchsichtige Streifen, welche keine Verbindungen unter einander zeigten und am Kopfe und Schwanze sich nicht weiter verfolgen ließen. . . . . Es erinnern diese gefäßartigen Streifen an ein ähnliches Organ bei den Nematoden.«

Claparède (6 and 7) held the Dicyemids to be Infusoria, nearly allied to the *Opalinidae*.

P. J. Van Beneden (13) finds resemblance between them and the *Gregarinae*.

Lankester (8, p. 96) says, »they are clearly not Infusoria, but a degraded form of worm, being multicellular in structure«. This opinion is still adhered to in his »Notes on Embryology and Classification«, p. 44 and 45.

Huxley (35), without distinctly pledging himself to the view maintained by Ed. Van Beneden, says, »Prof. E. Van Beneden has proved that these parasites cannot be dismissed, sans façon, as retrogressively metamorphosed 'worms'; and though I am not disposed to attach much weight to the absence of a mesoderm, on which Van Beneden insists as a distinction between the *Dicyemida* and the

Metazoa, the manner in which the contents of the axial cell give rise to germs is so completely unlike anything which is known to obtain in the *Metazoa*, as, to my mind, to justify the separation of the *Dicyemida* from the whole of this division.« p. 676.

Gegenbaur (31, p. 257) has expressed the opinion that the Dicyemids belong to the »Entwickelungskreise von Plattwürmern (Cestoden oder Trematoden).« In the last edition of his '*Grundriss d. vergl. Anat.*', he regards the Dicyemids as simple Metazoic forms, maintaining that the chief distinction between a Metazoon and a Protozoon lies, not in the plurality of cells, but in the arrangement of the cells in layers of different functional value. »Der Organismus von Dicyema zeigt sich somit als ein zweischichtiger mit functioneller Scheidung der beiden Schichten, davon die innere die morphologisch geringste Differenzirung besitzt, indem sie nur aus einer einzigen Zelle besteht. Ob darin ein primitiver Zustand sich ausspricht, ist jedoch desshalb nicht völlig sicher, weil die parasitische Existenz der Dicyemen die Rückbildung eines mehrzelligen Entoderms bedingt haben kann.« p. 72—73.

Giard (15, p. 461) thinks there can be no doubt that both the *Orthonectidae* and the *Dicyemids* belong to the phylum of the Vermes. With reference to the question of degeneration, and the position to be assigned to these two classes of parasites in the genealogical table, he remarks, — »Les *Orthonectida* doivent occuper dans ce tableau une place inférieure aux *Dicyemida*. Ces derniers sont évidemment très dégradés par le parasitisme. Leur organisation a dû être autrefois bien plus élevée qu'elle ne le paraît aujourd'hui. Le tégument renferme d'une façon très évidente (*Dicyema* de la Seiche) les bâtonnets si caractéristiques de la peau des Turbellariés, et l'embryon présente un organe très complexe, l'urna; rien de pareil ne s'observe chez les *Orthonectida*.«

Balfour (22, p. 111) observes, — »Till the further development of the infusoriform embryo is known, it is not possible to arrive at a definite conclusion as to the affinities of this strange parasite. Van Beneden is anxious to form it, on account of its simple organisation, into a group between the Protozoa and the Metazoa. It appears however very possible that the simplicity of its organization is the result of a parasitic existence; a view which receives confirmation from the common occurrence of the process of endogenous cell-formation in the axial hypoblast cell. It has been clearly shewn by Strasburger that endogenous cell-formation is secondarily derived from cell-division; so that

the occurrence of this process in Dicyema probably indicates that the hypoblast was primitively multicellular.«

With reference to the attempt to set up a mesozoic type for the reception of the Dicyemids, Claus (26, p. 201) speaks as follows; — »Indessen scheint diese Deutung der höchst merkwürdigen Dicyemen gewissermaßen als rückgebildete Gastraeaden mit einer einfachen Entodermzelle durchaus ebenso hypothetisch, als die Aufstellung eines Mesozoentypus auf Grund des Dicyemidenorganismus willkürlich und unhaltbar.«

Metschnikoff (19, p. 299) thinks that the Dicyemids and Orthonectidae are not very closely related; but regards both as degraded worms, and calls attention to the similarity between the Orthonectidae and Dinophilus in respect to a very pronounced sexual dimorphism.

Leuckart (13, p. 95—96) holds that both classes of parasites are closely related, and that they can be included among the Trematodes. After describing the embryos of *Distomum hepaticum*, he says, — »Die Würmchen, welche ich hier beschrieben habe, erinnern in so vieler Hinsicht an die von Giard und Metschnikoff bei Ophiuren und Turbellarien beobachteten Orthonectiden, dass ich kein Bedenken trage, diese merkwürdigen, mehrfach, wie die verwandten Dicyemiden, als Übergangsformen der Protozoen zu den vielzelligen Organismen betrachteten Schmarotzer unmittelbar an unsere Embryonen anzuknüpfen und der Trematodengruppe zuzuweisen. Dass dieselben niemals über den Embryonalzustand hinaus sich entwickeln, vielmehr Zeitlebens in diesem verharren und durch geschlechtliche Differenzirung der Keimzellen zu männlichen und weiblichen Individuen werden, kann uns in dieser Auffassung um so weniger beirren, als die geschlechtsreifen Entozoen der niedern Thiere, wie ich das an einem andern Orte des Nähern aus einander gesetzt und in seinen Consequenzen dargelegt habe[1], ihrem morphologischen Werthe nach fast sämmtlich auf mehr oder minder weit entwickelte Jugendformen sich zurückführen lassen.«

Nearly all the authorities thus far cited concur in the opinion that the Dicyemids are worms, and regard them as degenerate or arrested forms, which may be definitive, or only one of a cycle of generations. Quite an exceptional position has been taken by Ed. Van Beneden (1 and 2); and although his views have not, so far as I am aware, been accepted by any one except his pupil, Julin, they are certainly entitled

[1] 'Parasiten', 2. Aufl. 1880. Bd. I, p. 149.

to special consideration, inasmuch as they are based on personal observations of a very important character. According to Van Beneden, the Dicyemids can not be included in either of the two great divisions of the animal kingdom. They are not *Protozoa*, because they are pluricellular, and develop from a single cell by a process of division; nor, since they never develop more than two germ-layers, can they be *Metazoa* which are all triploblastic. The Dicyemids, says Van Beneden, show no trace of a mesoderm; and if they were included in the Metazoic division of animals, »il faudrait modifier la définition du Métazoaire. Si même, faisant abstraction de ce caractère, on recherche s'il existe parmi les Métazoaires un groupe qui, soit à raison de son organisation, soit par son développement, présente quelques affinités avec les Dicyémides, on arrive à une conclusion négative . . . . . L'organisation des *Dicyema* est beaucoup plus simple que celle de tous les Métazoaires connus: ils sont formés d'un fort petit nombre de cellules accolées entre elles et vivant ensemble pour former une individualité de second ordre. Ils ne possèdent aucun organe différencié ni aucune cavité interne. De ce chef, les Dicyémides sont inférieurs aux Metazoaires. Leur pluricellularité les élève au-dessus de tous les Protozoaires: il convient, ce me semble, de leur donner une place intermédiaire et de créer pour eux un embranchement des *Mésozoaires*.

»Nous sommes conduits à la même conclusion si nous prenons en considération le développement de l'embryon vermiforme des Dicyémides. A un moment donné de son évolution ontogenique, le Dicyémide est une véritable *Gastrula*, formée par épibolie, chez laquelle l'endoderme est représenté par une cellule unique. L'organisme complétement développé n'est que cette même *Gastrula* agrandie, chez laquelle le blastopore s'est fermé. . . . . La Gastrula des Dicyémides est comparable à cette *Gastrula* épibolique des poissons osseux. Chez un Dicyémide l'endoderme reste constitué par une cellule unique, pendant toute la durée de la vie.

»Les Dicyémides sont donc construits sur le type de la *Gastrula*, et comme cell-ci apparaît dans le cours de l'évolution des Métazoaires avant cette autre forme qui se caractérise par l'existence de trois feuillets cellulaires, il est clair que les Dicyémides sont inférieurs aux Métazoaires et ils justifient tant au point de vue de l'évolution qu'au point de vue de l'organisation l'etablissement d'un embranchement des *Mésozoaires*.« (1, p. 91—93.)

In his Contribution to the Natural History of the Dicyemids, just published, I find no retraction of any of the above statements. Although

recognizing the fact established by the HERTWIG's (31), that the formations which arise between the ectoderm and endoderm do not have the same morphological value by all Metazoa, VAN BENEDEN still maintains that the Dicyemids are clearly distinguished from each of the three metazoic divisions, *Coelenterata*, *Pseudocoelia*, and *Enterocoelia*, since they present neither mesenchym nor coelomic lamellae, nor even a structureless layer of any description between the ectoderm and endoderm.

As to the possibility of degeneration by parasitism, we are told that »dans le développement d'un Dicyémide, rien n'indique une dégénérescence et le fait du parasitisme ne suffit pas pour affirmer une rétrogradation«. (p. 219.)

JULIN's interesting researches on the Orthonectidae (21) have led him to the conclusion that these parasites are very closely allied to the Dicyemids, as shown both by their developmental history and their adult structure. As to the necessity, or propriety, of establishing a middle, or mesozoic division of the animal kingdom, JULIN adopts, without reserve, the opinion of VAN BENEDEN, claiming that the Orthonectidae as well as the Dicyemids are diploblastic animals, and as such must be separated from the Metazoa.

VAN BENEDEN (2) has described two rare and peculiar forms, *Conocyema polymorpha* from *Octopus vulgaris*, and *Microcyema vespa* from *Sepia officinalis*, to which he has given the family name *Heterocyemidae*. The Dicyemidae and the Heterocyemidae unite to form the order, *Rhombozoa*; and the *Rhombozoa* with the collateral order, *Orthonectida* constitute the new type, *Mesozoa*.

Such is the imposing edifice of names placed on the backs of two minute parasites whose life-history is still, in some of its chief features, a complete enigma. Although our knowledge of the Dicyemids and the Orthonectidae is not sufficiently complete to enable us to point out with certainty their proximate allies, it is nevertheless ample enough for forming a tolerably clear notion of their more remote affinities. VAN BENEDEN contends that these animals cannot be included among the Metazoa, without modifying the definition of the latter. One might ask, what possible objection can there be to a modification of this definition? So far as the mesoderm is concerned, the necessity for a modification has already arisen; and I see no reason why the definition of *Metazoa* should be less elastic than that of *Mesozoa*, which has just been remodeled for the reception of the *Orthonectidae*. Every question of this kind has at least two sides, the theoretical and the practical. The idea of a mesozoic type is, in itself, unobjectionable. No one will deny that there

is a gap between *Protozoa* and *Metazoa* as now defined: and no one could object to seeing it filled. Between the homoblastic and the diploblastic[1] condition there is a distance that has not yet been measured; and I think few will deny that it is immensely greater than that which now separates the triploblastic from the diploblastic state. The nature of this gap has been indicated by Gegenbaur, in placing the distinction between *Protozoa* and *Metazoa*, not in the number of cells, but in the arrangement of the same in distinct layers of different functional value. From this point of view it is obvious that the hiatus can not be filled by such animals as the Dicyemids and Orthonectids.

Van Beneden denies the existence of a mesoderm in either of these parasites, and on this ground alone attempts to justify the creation of a new type. Even if this denial were well founded — and I believe it is not — diploblastic animals could not bridge over the gulf between *Protozoa* and *Metazoa*: and if a new type be admitted for such animals, what would be done in the event of the discovery of a Blastula?

If diploblastic animals be defined as animals composed of two cell-layers, and triploblastic animals as having three distinct cell-layers, the distinction between the two would certainly be great; but the moment we insist on extending the meaning of the word mesoderm so as to include the *mesenchym* and a *gelatinous lamella* entirely free from cells, we virtually acknowledge that no broad line of distinction can be maintained. Now this is precisely what Van Beneden has been compelled to do in order to bring his views into harmony with the conclusions reached by the Hertwigs. If, on the other hand, we adhere to the definition of the mesoderm as a cell-layer which arises between the ectoderm and endoderm during the earlier stages of development — not as the result of an ulterior histological differentiation — then it becomes necessary to include both diploblastic and triploblastic animals in the metazoic group, and the idea of a didermic Mesozoon is left without »a local habitation«. It appears to me then — even on the supposition that these parasites are strictly diploblastic — there are no adequate grounds for separating them from the Metazoa: and it is quite impossible to allow that they represent forms »*qui ont fait la transition entre les Protozoaires et les Métazoaires*«.

But is there no trace of a mesoderm in the so-called Mesozoa? I

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[1] This terminology is borrowed from Lankester. »On the Germ. Layers« etc. 'Ann. Mag. Nat. Hist.' 1873.

fail to see what better evidence of the existence of such a lamella could be brought than has already been produced by JULIN; and I can not therefore accept the interpretation which he has given to the »cellules intermediaires«. On this point JULIN remarks, that »we find in certain Metazoa a formation quite similar to that of the muscular layer of the Orthonectidae, — a formation which is, however, completely independent of that of the mesoderm and has nothing to do with the latter. The muscles of the Orthonectidae consist only in a histological differentiation of the superficial layer of the endoderm. It is something analogous to what takes place, according to the observations of the HERTWIGS, in the formation of the muscular tissue of the Actiniae. Here also, 'es sind allein die epithelialen Begrenzungsschichten, aus welchen sich die für die höhere Entwicklung des Organismus so überaus wichtigen Elemente, die Muskel-, Nerven- und Sinneszellen differenziren' (34), and this formation has nothing in common with the mesoderm, which consists of a mesenchym composed solely of a gelatinous substance«. (21, p. 42.) Then comes the following important statements. »La seule différence, qui existe entre les éléments musculaires des Actinies et ceux des Orthonectides, consiste en ce fait que, tandis que chez les premiers ces éléments restent, pendant toute la durée de la vie, en continuité directe avec les cellules endodermiques qui leur ont donné naissance, chez les seconds, au contraire, ces éléments se distinguent des autres cellules endodermiques à une phase plus ou moins reculée du developpement. Cette différenciation d'une partie des cellules endodermiques en cellules musculaires se fait beaucoup plus tard chez la femelle que chez le mâle de *Rh. Giardii*.« (p. 42—43.)

To my mind, JULIN has shown conclusively that the Orthonectidae have a veritable mesoderm, — a distinct cell-layer, arising between the entoderm and ectoderm at an early period of the development, not as the result of a histological differentiation, but prior to and independent of such differentiation. In the male, these cells arise at the two poles of the endodermic cell; and the fact that they do not form a complete envelope around the central cell or cells until after they have undergone a fibrous differentiation is of course no objection whatever to the view I have taken. In the case of the female, the mesoderm (»couche superficielle de l'endoderme«, JULIN) forms a complete envelope around the endoderm (»masse endodermique centrale«, JULIN), and this long before the appearance of any muscular differentiation. To affirm that this cell-layer is not a mesoderm, and at the same time maintain that

a gelatinous lamella entirely devoid of cells is a mesoderm, is the somewhat singular position now occupied by Van Beneden and Julin. That Julin's denial of a mesoderm is not easily reconciled with his account of the origin of the muscle-fibres, has, if I mistake not, been quickly perceived by Van Beneden. My ground for so inferring is the fact that Van Beneden has not allowed these statements to pass without a demurrer. »Il est à remarquer,« says Van Beneden, »que rien dans les recherches de M. Julin, ne prouve que cette couche cellulaire engendre les fibrilles, loin d'établir qu'elle est tout entière employée à la formation de ces fibrilles. . . . . Si réellement, ce qui ne me paraît pas prouvé, ces fibrilles sont des dépendances de ces cellules, il me semble bien plus probable que celles-ci, après avoir donné naissance aux fibrilles et avoir affecté temporairement le type épithélio-musculaire, se transforment en oeufs tout comme les cellules axiales.« (2, p. 221.)

According to this view, the endoderm of the Orthonectidae differentiates early into two distinct parts, a central mass of cells and a superficial cell-layer interposed between the central mass and the ectoderm. The entire central portion is converted into sexual products: the superficial layer imitates for a short time an epithelio-muscular layer; but it maintains this character only transiently, for its epithelial portion soon becomes sexual cells, and the persistent muscular portion thus becomes an independent layer. Julin's view differs from this only in one point: he holds that the entire superficial layer is converted into the muscular layer. Van Beneden finds it difficult to accept Julin's conclusion in this particular, since the muscular layer is exceedingly thin, while the superficial layer itself is very thick. »How can we admit,« he asks, »that a layer so thick as the range of cells subjacent to the epidermis of larvae like those which are figured (Pl. 3, figs. 8—13. Julin), is wholly employed in the formation of these fibrils?«

For my part, I see nothing incredible in the opinion that this entire superficial layer, after becoming gradually thinner by a process of atrophy, should eventually take the form of a thin muscular layer. It is of course possible that only a part of its cells assume the muscular form, the rest becoming sexual products. Either of these views seems to me more probable than the ingenious theory of a transient epithelio-muscular stage. The assumption of the reproductive function by a part of these cells is a possibility which recalls our attention to a point incidentally mentioned before. I have compared the two primary germ-cells in the vermiform embryo of a Dicyemid to the two primary cells that arise at the poles of the central cell of the male Orthonectid.

In both cases these cells arise from the two poles of the central or axial cell. In the Dicyemids they lose their polar position and wander into the central cell; in the Orthonectidae, they maintain their original position. The fact that these polar cells become muscle-fibrils in one case, and germ-cells in the other, is no objection to the opinion that they are morphological equivalents. It is on this ground that the *Dicyemids* may be said to have a transient triploblastic stage, represented by an ectodermal layer, an axial endodermic cell, and two intermediate mesodermic cells derived from the two poles of the endodermic cell.

In the Orthonectidae there is a great reduction of the mesoderm by atrophy, or otherwise, during the course of development; and there can be little doubt that this reduction stands in correlation with an increased reproductive activity. In the Dicyemids we see an abridgment of the same process in the fact that no permanent mesoderm ever comes to development.

Thus far no objection has been raised to the creation of a middle division of the animal kingdom on the ground that all the assumed representatives of this division are parasites. But I think this must be admitted to be one of the unfortunate aspects of the case, although we may not be able to point to undeniable evidence of degeneration. But who will venture to assert that, before applying the hypothesis of »degenerative evolution«, it is indispensable to find unmistakable marks of degeneration, such as are seen, for example, in the development of *Sacculina*, *Lernaeocera*, *Barnacles*, etc.? That this hypothesis admits of a very wide application to the simpler forms of life has been made sufficiently clear by Dohrn (27) and Lankester (40). When we find an animal in the form of a simple sack, filled with reproductive elements, secured by position against enemies, supplied with food in abundance, and combining parasitism with immobility, we have strong reasons for believing that the simplicity of its structure is more or less the result of the luxurious conditions of life which it enjoys, even if its development furnishes no positive evidence of degeneration.

As to the systematic position of the Dicyemids, I see no good reason for doubting the general opinion that they are Plathelminths degraded by parasitism. Whether they and their allies, the Orthonectidae, have descended from ancestors represented now by such forms as *Dinophilus* (Metschnikoff), or from the *Trematoda* (Leuckart), is a question which further investigations must decide.

Résumé.

1. According to the method of classification introduced by Ed. Van Beneden, the generic and specific names of the Dicyemids (Heterocyemids excluded) run parallel with those of their hosts, genus for genus and species for species. The Dicyemids found in four genera of Cephalopods, *Octopus*, *Eledone*, *Sepia* and *Sepiola*, were divided into four corresponding genera, *Dicyema*, *Dicyemella*, *Dicyemina*, and *Dicyemopsis;* and these four genera were said to include seven species corresponding to the same number of species of Cephalopods. The correspondence here assumed as a basis of classification is disproved by the following facts:

(1) In Eledone moschata are found two species of Dicyemids, *Dicyema moschatum* and *Dicyemennea Eledones;* and in Sepia officinalis, likewise two species, *Dicyema truncatum* and *Dicyemennea gracile*.

(2) *Dicyemennea Eledones* occurs in two different Cephalopods, Eledone Aldrovandi and Eledone moschata; and *Dicyema truncatum* is found in three Cephalopods, Sepia officinalis, Sepia elegans, and Rossia macrosoma.

(3) Two closely allied species of Dicyemids, *Dicyemennea Eledones* and *Dicyemennea gracile*, are found in Cephalopods belonging to two different families; while two widely different species of Dicyemids, *Dicyema truncatum* and *Dicyema macrocephalum*, occur, singly, in two closely related genera of Cephalopods (Rossia and Sepiola).

2. The ten species of Dicyemids decribed on pages 9—29 represent two genera distinguishable by the number of cells in the head, or calotte.

The genus *Dicyema* Köll., including seven species, has a bilateral calotte composed of eight cells (octamerous), in two sets of four each (propolar and metapolar). The calotte is invariably *orthotropal* (p. 8) in the embryo, but in the adults of most species is generally more or less oblique (*plagiotropal*, p. 8—9 and 11). The propolars are always two dorsal and two ventral, and are *opposite* the corresponding metapolar cells, except in *Dicyema truncatum* where the metapolars are two lateral, one dorsal, and one ventral (*alternate* order, p. 21—22).

The genus *Dicyemennea*, including three species, has a bilateral calotte constituted of nine cells (enneamerous, p. 9), four propolar and five metapolar. The propolars are two dorsal and two ventral, and

always much smaller than the three dorsal (one median and two lateral) and two ventral metapolars (p. 9 and 26—27). Calotte permanently orthotropal.

The parapolars (p. 8) are two and lateral, in both genera. The caudal cells are always two, and dorso-ventral, at least in the youngest individuals.

3. The dorsal and ventral sides of the calotte are not distinguishable by any uniform difference in the size of the polar cells. In Dicyemennea, the three dorsal metapolars serve as a means of orientation. In Dicyema, the plagiotropal calottes face the ventral side; the sides of the orthotropal calottes are determined by the relative position of the parapolars and the adjoining ectodermal cells (p. 10).

4. The obliquity of the octamerous calotte and the inequality of its cells are extremely variable among individuals of the same species; and the range of variability can seldom, if ever, be ascertained from examples taken from a single Cephalopod (p. 12, 17, 21). It is necessary therefore to discriminate carefully between *individual* peculiarities and *specific* characters (p. 2).

The ectoderm furnishes all the diagnostic characters: the whole number of ectodermal cells, the length of adult individuals, the size of the polar cells both relative and absolute, their shape, position, and axial relations, the form of the parapolar and caudal cells, etc., supply the means of distinguishing species.

5. When two species occur in the same Cephalopod, they may occupy separate renal chambers and thus be completely isolated; or they may be cohabitants of the same chambers. In the latter case they are seldom promiscuously associated, but distributed in colonies (p. 14, 28).

If only one species is present, a colonial distribution is often observable, some renal lobes being occupied more or less exclusively by extremely long individuals, others by shorter individuals. Sometimes the entire renal organ, with the exception of one or two lobes will be free from the parasite. We may infer from these facts that they are not introduced into the host in large numbers at any one time.

6. The Dicyemids may be divided, according to the share they take in the work of reproduction, into *monogenic* and *diphygenic* (or diplogenic) individuals (p. 55). The first class (primary Nematogens) (p. 36), produce only vermiform embryos; the second produce first infusoriform embryos, then vermiform embryos (secondary Nematogens) (p. 41). Rhombogen and secondary Nematogen denote two different phases in the life of the same individual. It is still doubtful whether the monogenic and

diphygenic individuals are heterogeneous forms. They are alike in origin, development, and adult form and structure; but their germ-cells pursue different courses of development, either because, contrary to appearances, they are fundamentally unlike, or as the result of conditions that differ in some unknown respects (fecundation? food? position in a cycle of generations? seasonal influences?) Cf. p. 61.

7. There is a remarkable correspondence between the age of the host and the reproductive phenomena of the parasite. Nematogenic individuals are abundantly represented in young Cephalopods (p. 33, 59), sometimes exclusively; while in adult Cephalopods Rhombogenic individuals are generally very numerous, and, in rare cases, appear to be the sole occupants of the renal organ (p. 45). This fact may be interpreted in favor of a cyclical occurrence of Rhombogenic reproduction (p. 34), but can hardly be said to be analogous to the isolated occurrence of male and female Orthonectidae. Cf. Julin, 21, p. 44; also Van Beneden, 2, p. 209.

8. The fact that the same individual (diphygenic) may produce consecutively infusoriform and vermiform embryos, is substantiated by a variety of confirmatory phenomena, most of which are connected, historically, with the origin of free nuclei in the axial cell (p. 34—47).

The proof that some individuals (monogenic) produce throughout only vermiform embryos, lies in the fact that adult Nematogens are found in which the axial cell contains but one nucleus.

9. The Rhombogenic mode of reproduction alone gives rise to a plurinucleated axial cell. There are two classes of free nuclei appearing in the axial cell in addition to the large central nucleus of this cell itself. The first class are, conjecturally, of the nature of polar globules. Each germ-cell, before developing into an Infusorigen, eliminates one such corpuscle (*n'*). These bodies (»paranuclei«) agree in all their features with nuclei, and are therefore called »free nuclei«. The largest number of such nuclei observed in any one case is eight, corresponding to the number of Infusorigens. In pure Rhombogens, i. e. Rhombogens that have not entered upon transitional phases, the whole number of free nuclei — counting the original central nucleus — always exceeds by one the number of Infusorigens.

The second class of free nuclei are the »*residual nuclei*« (*n''*) of the germogens (*c*) set free, as the final event in the history of the Infusorigens. As there may be from one to eight Infusorigens in the same individual, and as one free nucleus marks the beginning, another the end, of each Infusorigen, it follows that a diphygenic Dicyemid may

have, during its Rhombogenic period, from 2—9 free nuclei, and during the subsequent Nematogenic period, any odd number from 3—17 inclusive. (Cf. p. 39.)

10. The Infusorigen is a group of cells consisting, at one period (fig. 109), of a peripheral layer of cells partially enveloping a large central cell. Its development from a single cell by a process of cleavage, and the epibolic growth of its peripheral layer, give ground for thinking that the Infusorigen represents an individual, comparable with the Gastrula of a vermiform embryo. The peripheral layer may be compared to the ectoderm, the central cell to the axial cell, and the »residual nucleus« to the »central nucleus«. Germ-cells arise endogenously both in the central cell (germogen) and in the axial endodermic cell. For general considerations arising out of this interpretation, consult pp. 48—54, 59—61.

11. The two kinds of embryos produced by diphygenic individuals arise from two distinct kinds of germ-cells, both of which originate, in succession, in the Infusorigen. First in order, arise the germ-cells destined to develop into infusoriform embryos. These infusorific cells are comparatively large (.016 mm) and have large nuclei (.01—.012 mm). They are set free in small numbers, one or two at a time (p. 54). At length the production of this kind of cells ceases, the infusoriform embryos escape, and nothing remains of the Infusorigen except the germogen (fig. 112, *c*) and a number of small loose cells (about .008 mm in diam.) with small nuclei (.004—.005 mm). These cells multiply by division until they fill the larger portion of the axial cell, and eventually give rise to vermiform embryos. The Rhombogenic period is thus separated by a considerable interval (transitional period, p. 41) from the Nematogenic.

It very seldom happens that the development of vermiform embryos begins *before* the infusoriform embryos have all escaped (p. 42—43).

12. Developmental division (cleavage) and multiplicative division are displayed in striking contrast in the history of diphygenic individuals. Simple division gives rise to from 2—8 scattered germ-cells; cleavage produces a diploblastic group, the Infusorigen; infusorific and vermific germ-cells are then produced by division; and these in due time undergo developmental division. The alternation between division and cleavage is twice repeated. The structural unity of the Infusorigen is attested by the cohesion of its cells. It has a developmental and a reproductive period. (Cf. pp. 40, 53.)

13. No definite evidence of fecundation has been obtained; but it

is not improbable that one of the two modes of reproduction exhibited in diphygenic individuals may be introduced by fecundation (p. 58—59).

In four instances modified forms of Infusoriforms have been found in Nematogens (p. 59). Although it is quite certain that these Infusoriforms did not originate in the Nematogens, it is not clear whether they are to be regarded as stragglers, or males (Van Beneden) that had penetrated for the purpose of fecundating. The former supposition appears to me the more probable.

14. The first cleavage, introduced karyokinetically, splits the vermific germ-cell into two unequal parts. The two-cell stage is followed by one of three cells, and this by one of four. Three of the four cells form a cap to the fourth (fig. 71), and gradually envelope it by epibolic expansion. A Gastrula is thus formed consisting of a small number of ectodermic cells and a single central endodermic cell (figs. 76 and 77). Periods of growth intervene between the successive cleavages, the individual cells attaining, each time, approximately the size of the original germ-cell.

The blastopore closes, and the multiplication of cells at this pole soon leads to the pyriform embryo (figs. 80, 81, 87). The pointed end of this embryo is an elongation from the blastoporal region; the broad end corresponds to the future cephalic pole. The endodermic cell lies at first wholly in the broad end, but gradually elongates backward between the caudal cells. The whole number of cells seen in the adult are present before the embryo attains its definitive form (p. 63).

15. Two primary germ-cells arise at the two poles of the endoderm, the first (always the posterior) about the time the pyriform stage is reached (fig. 86). The nucleus of the endoderm participates only in the formation of these two cells, which give rise to other germ-cells by division. The manner in which the germ-cells arise in the germogen has not been ascertained; but it may be safely assumed that the nucleus of the germogen participates in their formation, at least in the first instance. For remarks on endogenous cell-formation, see p. 65.

16. Comparing the development of the vermiform embryo with the development of the male Orthonectid (Julin), Dicyemids may be said to have a transient triploblastic stage, represented by an ectodermal layer, one axial endodermic cell, and two mesodermic cells (primary germ-cells) derived from the two poles of the endoderm. (Vid. p. 75.)

Literature.

Dicyemidae.

1. **Ed. Van Beneden.** »Recherches sur les Dicyémides, survivants actuels d'un Embranchement des Mésozoaires.« Extrait des 'Bull. de l'Acad. roy. de Belgique', 2me sér., t. XLI, no. 6, et t. XLII, no. 7, 1876.
2. —— »Contribution à l'histoire des Dicyémides.« 'Archives de Biologie', t. III, f. I, p. 195, 1882.
3. —— »Prof. Ed. Van Beneden's Researches on the Dicyemidae.« Abstract by Mr. D'Arcy Power, in the 'Quart. Journ. Mic. Sci.', vol. XVII (N. S.), p. 132, 1877.
4. **Kölliker.** »Über Dicyema paradoxum, den Schmarotzer der Venenanhänge der Cephalopoden.« 2ter Bericht d. königl. zootom. Anstalt zu Würzburg', p. 59, 1849.
5. **Wagener, G. R.** »Über Dicyema Kölliker.« 'Müll. Arch.', p. 354, 1857.
6. **Claparède, Ed.** and **Lachmann.** »Etudes sur les Infusoires et les Rhizopodes.« t. II, p. 201, 1860—61.
7. **Claparède, Ed.,** »Zusatz« to Wagener's paper (no. 5).
8. **Ray Lankester.** Summary of Observations made at Naples in the winter of 1871—72. p. 95—96. 'Ann. and Mag. Nat. Hist.', 4th ser., vol. XI, 1873.
9. **Erdl.** »Über die beweglichen Fäden in den Venenanhängen der Cephalopoden.« 'Archiv f. Naturgesch.' Bd. I, p. 162—167, 1843.
10. **Krohn.** »Über das Vorkommen von Entozoen und Krystallablagerungen in den schwammigen Venenanhängen einiger Cephalopoden.« Froriep's 'Neue Notizen' Bd. XI, No. 234, p. 214, 1839.
11. **Leuckart.** »Bericht üb. d. wiss. Leist. d. nied. Thiere« 1876—1879, p. 210—215. 'Arch. f. Naturgesch.' 24, Bd. II, p. 594—599.
12. **Siebold.** »Bericht über die Leistungen im Gebiete der Helminthologie während des Jahres 1843 u. 1844.« 'Arch. f. Naturgesch.' 1845, p. 247.
13. **Van Beneden, P. J.** »Commensaux et Parasites.« Paris, Germer Baillière. 1875.

Orthonectidae.

14. **Giard.** »Sur les Orthonectida, classe nouvelle d'animaux parasites des Échinodermes et des Turbellariés.« 'Compt. rend.', Oct. 29, 1877.
15. —— »Les Orthonectides, nouvelle classe du Phylum des Vermes.« 'Journ. de l'anat. et de la physiol.' t. XV, 1879. Also in 'Quart. Journ. Mic. Sci.' vol. XX.
16. —— »Nouvelles remarques sur les Orthonectida.« 'Compt. rend.', Dec. 15, 1879, and 'Zool. Anzeiger', 1880.
17. **Metschnikoff.** »Zur Naturgeschichte d. Orthonectiden.« 'Zool. Anz.' No. 40, p. 547, 1879.
18. —— »Nachträgliche Bemerkungen üb. Orthonectiden.« 'Zool. Anz.' No. 43, p. 618, 1879.
19. —— »Untersuchungen üb. Orthonectiden.« 'Zeitschr. f. wiss. Zool., Bd. XXXV, 1881.
20. **Julin.** »Observations sur le Développement des Orthonectides.« 'Bull. sci. du départ. du Nord', 2e sér. 4e année, nos 10—11, 1881.
21. —— »Contribution à l'histoire des Mésozoaires.« 'Archives de Biologie', t. III, f. 1, p. 1—49, 1882.

Further References.

22. **Balfour.** »Comparative Embryology.« Vol. I, p. 108—111.
23. **Van Beneden, Ed.** »Recherches sur l'evolution des Grégarines.« 'Bull. de l'Acad. roy. de Belg.', 2me sér., t. XXXI, no. 5, 1871.
24. **Brock.** »Zur Anat. u. Syst. d. Cephalopoden.« 'Zeitschr. f. wiss. Zool.' XXXVI, p. 543, 1882.
25. **Carus.** »Zur näheren Kenntnis des Generationswechsels.« Leipzig, 1849.
26. **Claus.** »Grundzüge d. Zool.« 4te Aufl. Bd. I, p. 201, 1880.
27. **Dohrn.** »Der Ursprung d. Wirbelth. u. d. Princip d. Functionswechsels.« Leipzig, 1875.
28. **Drasch.** »Die physiol. Regener. d. Flimmerep. d. Trachea.« 'Sitzb. d. k. Akad. d. Wissensch.' III. Abth. LXXX, Oct., 1879, and LXXXIII, 1881.
29. **Foettinger.** »Recherches sur Quelques Infusoires Nouveaux, Parasites des Cephalopodes.« 'Arch. de Biol.' vol. II, p. 345, 1881.
30. **Fraipont.** »Recherches sur les Acinétiniens de la Cote d'Ostende.« 'Bull. de l'Acad. roy. de Belg.', 2me sér., t. XLIV, No. 12, 1877 and t. XLV, No. 3 & 4, 1878.
31. **Gegenbaur.** »Vergl. Anat.« p. 257, 1870.
32. —— »Grundriss d. vergl. Anat.« 2te Aufl. p. 72—73, 1878.
33. **Hertwig, R.,** »Beiträge z. Kenntniss d. Acineten.« 'Inaugural-Diss.' Leipzig, 1875.
34. **Hertwig, O. & R.** »Die Coelomtheorie.« 'Jen. Zeitschr.' Bd. XV, p. 1, 1881.
35. **Huxley.** »A Manual of the Anat. of Invert. Animals.« 1877.
36. **Iwakawa.** »The Genesis of the Egg in Triton.« 'Quart. Journ. Mic. Sci.', vol. XXII, p. 259, 1882.
37. **Kleinenberg.** »Sull origine del sistema nervoso centrale degli Anellidi.« 'Mem. R. Accad. dei Lincei', 1880—1881. (3). Vol. X. Reviewed by J. W. Spengel, 'Biol. Centralbl.' p. 231—236, 1882.
38. —— »Über die Entstehung d. Eier bei Eudendrium.« 'Zeitschr. f. wiss. Zool.' Bd. XXXV, p. 326, 1881.
39. **Korschelt.** »Über Bau u. Entw. d. Dinophilus apatris.« Vorl. Mitth. 'Zool. Anz.' No. 117, Bd. V, p. 398, 1882.
40. **Lankester.** »Degeneration. A Chapter in Darwinism.« Nature Series, London, 1880.
41. —— »Notes on Embryology and Classification for the Use of Students.« London, 1877.
42. —— »Observations on the Development of the Cephalopoda.« 'Quart. Journ. Mic. Sci.', XXIII, 1875.
43. **Leuckart.** »Zur Entw'gesch. d. Leberegels etc.« 'Arch. f. Naturg.' XXXXVIII. Jahrg. Bd. I.
44. —— »Die Parasiten d. Menschen.« 2te Aufl., Bd. I, 1879.
45. **Mark.** »Maturation, Fecundation, and Segmentation of Limax campestris, Binney.« 'Bull. Mus. Comp. Zool.' Cambridge. Vol. VI, P. II, No. 12, p. 173, 1881.
46. **Siebold.** »Gyrodactylus, ein ammenartiges Wesen.« 'Zeitschr. f. wiss. Zool.' Bd. I, p 347, 1849.
47. **Strasburger.** »Neue Beobacht. üb. Zellbildung und Zelltheilung.« 'Botan. Zeitung', Leipzig, 1879.
48. —— »Zellbild. u. Zelltheilung.« 3te Aufl., 1880.

49. **Wagener, G.** »Über Gyrodactylus elegans von NORDMANN.« 'Arch. f. Anat. u. Physiol.', p. 768, 1860.
50. **Weismann.** »Die Entsteh. d. Eizellen in d. Gattung Eudendrium.« 'Zool. Anz.', IV, p. 111, 1881. Comp. earlier papers in 'Zool. Anz.' 1880, p. 226 and 367.
51. **Whitman.** »Embryology of Clepsine.« 'Quart. Journ. Mic. Sci.', 1878.
52. **Vigelius.** »Über das Excretionssystem d. Cephalopoden.« 'Niederl. Arch. f. Zool.' V, 1880.

Explanation of Plates 1—5.

adp	Dorsal propolar cells.	*c*	Germogen.
avp	Ventral propolar cells.	*n''*	Nucleus of the germogen (residual nucleus).
pdp	Dorsal metapolar cells.		
pvp	Ventral metapolar cells.	*n'*	Paranucleus.
pp	Parapolar cells.	*n*	Nucleus.
e	Endodermic cell.	*ne*	Nucleus of the axial cell.
ec	Ectoderm.	*pg*	Primary germ-cells.
ve	Vermiform Embryo.	*vg*	Vermific germ-cells.
m	Mother-cell of the Infusorigen.	*ig*	Infusorific germ-cells.
g	The Infusorigen.		

Plate 1.

Figs. 1—15 (*Dicyema moschatum*) magnified 465 diam., except fig. 14 which is magnified 280 diam.

Fig. 1. Anterior portion of a primary Nematogen, 0.85 mm in length, seen from one side. In front of the central nucleus (*ne*) of the axial cell are a fully formed embryo (0.1 mm long) containing a central nucleus and two primary germ-cells (*pg*), and scattered vermific germ-cells (*vg*). Besides what is seen in the figure, this Nematogen contained in the posterior portion only a few germ-cells and a few early stages of vermiform embryos. Acetic acid.

Fig. 2. Portion of a Rhombogen (1.2 mm long) seen from ventral side. This Rhombogen contained one Infusorigen and various stages of infusoriform embryos, and two large nuclei, seen in the fig. One of these nuclei is the »central nucleus« of the axial cell, the other is a paranucleus. They are so nearly alike that it is impossible to say which is the central nucleus and which the paranucleus. Acetic acid.

Fig. 3. A helmet-shaped calotte of a Rhombogen (1.2 mm long), seen from the right side. This individual contained the same elements in its axial cell as fig. 2. The two round nuclei were lodged in the anterior end of the axial cell, as seen in fig. 13. Acetic acid.

Fig. 4. A calotte of a Rhombogen (3.40 mm long), seen from the left side. The ventral polar cells are here, as in most of these figures, larger than the dorsal cells. Contained many embryos and several free nuclei. Acetic Acid.

Fig. 5. A very oblique calotte of a Rhombogen (3.50 mm long), seen from the right side. Acetic acid.

Fig. 6. A bell-shaped calotte of a Rhombogen (2.1 mm long), from the dorsal side. The metapolars form a flaring collar to the elongated propolars. Others of a similar shape were found at the same time. Acetic acid.

Fig. 7. Calotte of a Nematogen (3.95 mm long), from the ventral side. Contained several embryos and numerous germ-cells. Only two free nuclei were seen. Acetic acid.

Fig. 8. Ventral view of the calotte of a Rhombogen (2 mm long). Two free nuclei in the cephalic end of the endoderm. Acetic acid.

Fig. 9. Dorsal view of a very oblique calotte of a Rhombogen (ca. 3.5 mm long). Acetic acid.

Fig. 10. Head of a Nematogen (2.7 mm long) from the side, showing an embryo (*ve*) partially liberated. Acetic acid.

Fig. 11. The calotte and parapolars of a primary Nematogen (1.9 mm long) from the ventral face. Contained one large nucleus, a few embryos, and many germ-cells. Acetic acid.

Fig. 12. Another Nematogen from the ventral face (length 1.5 mm). A central nucleus, germ-cells, and embryos. Acetic acid.

Fig. 13. A secondary Nematogen (.55 mm long) from the right side. The central nucleus and the paranucleus lodged in the head, and near them a granular body which represents the remains of a germogen. This individual has passed a Rhombogenic condition represented by a single Infusorigen. Acetic acid.

Fig. 14. A primary Nematogen from the left side (length 1.1 mm). Contained one vermiform embryo, a considerable number of germ-cells from .005 to .008 mm in diam., and a single nucleus. Shows the two verruciform cells in the hind half of the body. Acetic acid.

Fig. 15. A middle portion of a large *Dicyemennea Eledones*, in a transitional phase, showing the central nucleus (*ne*), two paranuclei (*n'*), numerous vermific germ-cells, and two Infusorigens in their last stage. The largest cells in the Infusorigens measure .016 mm in diam., the size of infusorific germ-cells. They have large nuclei (.01—.012 mm) while the vermific cells have small nuclei (.004—.005 mm). The nuclei of the smaller cells in the Infusorigens agree in size with those of the smaller vermific germ-cells. These small cells are probably derived from the larger ones with large nuclei by division. Acetic acid.

Plate 2.

Figs. 16—21 *Dicyema typus;* figs. 22—29 *Dicyema microcephalum.*

Fig. 16 magnified 280/1; fig. 22, 120/1; the remaining figs. 465/1.

Fig. 16. A young Rhombogen (.6 mm long) from the left side, showing all the ectodermal cells in situ. Parapolars followed by two dorsal and one ventral cell. Four granular verruciform cells. Osmic acid.

Fig. 17. A fully formed embryo (.25 mm long). Total number of ectodermal 25. Cells loosened by acetic acid. Contained a central nucleus and eight germ-cells.

Fig. 18. A portion of a Rhombogen as seen in a living condition. No distinct outlines of cells. Ectoderm shows minute round granules.

Fig. 19. Side view of a small Rhombogen (1 mm long). Anterior polar cells a little larger than the posterior. Osmic acid.

Fig. 20. Calotte of a Rhombogen (2.5 mm long) from one side. Propolars plainly larger than the metapolars. Acetic acid.

Fig. 21. A very oblique calotte of one of the longer Rhombogens (2.5 mm) from the dorsal side. Here the propolars are plainly smaller than the metapolars. Acetic acid.

Dicyema microcephalum (figs. 22—29).

Fig. 22. A Nematogen (1.2 mm long) filled with vermiform embryos. Head much more narrow than the body. Osmic acid.

Fig. 23. A young individual (.44 mm long) represented with all the cells in situ, from the ventral side. Parapolars adjoined by two dorsal and one ventral ectodermal cell. Total number of ectodermal cells 26. Osmic acid.

Fig. 24. A very small individual (.2 mm) with the cells loosened by acetic acid.

Fig. 25. Calotte, parapolars, and three ectodermal cells, seen from the left side. Osmic acid.

Fig. 26. The calotte of a Nematogen. Head broader than in the fore-going figures. Little difference in size between the propolars and metapolars. No obliquity in the calotte of this species.

Fig. 27. Cephalic portion of a living individual.

Fig. 28. Front view of the calotte.

Fig. 29. Dorsal view of a Nematogen. Cells somewhat swollen. Osmic acid.

Plate 3.

Figs. 30—34 *Dicyema Clausianum;* figs. 35—39 *Dicyema macrocephalum;* figs. 40—43 *Dicyema moschatum.*

Fig. 30 magnified 280/1; fig. 40, 1060/1; remaining figs. 465/1.

Dicyema Clausianum.

Fig. 30. A Rhombogen (1.4 mm long) from the dorsal side, with all the cells in position. Ectodermal cells 27. Three verruciform cells. Osmic acid.

Fig. 31. Calotte of a Rhombogen from the ventral side. Acetic acid.

Fig. 32. Optical section of the calotte of a large Rhombogen. The dorsal propolars form the tip of the very oblique calotte. Acetic acid.

Fig. 33. Ventral side of the fore end of a Rhombogen. Acetic acid.

Fig. 34. Side view of the calotte of a large Rhombogen (4 mm long). No obliquity Propolars larger than the metapolars. Acetic acid.

Dicyema macrocephalum figs. 35—39.

Fig. 35. Side view of a calotte, somewhat convex. Parapolars form here no part of the head.

Fig. 36. A portion of a Rhombogen (1.4 mm) seen from the right side (calotte in optical section). The two lateral parapolars followed by two dorsal and one ventral cell. Acetic acid.

Fig. 37. Face view of the calotte of a Rhombogen. The propolars form the central part of the cephalic disc, the metapolars its rim. Acetic acid.

Fig. 38. Calotte of a young Rhombogen (.7 mm long), in which the parapolar collar (*pp*) is seen to be composed of two cells. Propolars not visible. Osmic acid.

Fig. 39. Optical section of a young individual (.25 mm). Osmic acid.

Dicyema moschatum.

Fig. 40. A portion of a Transitional.

Fig. 41. Different forms of germ-cells found in a transitional. Some are undergoing division. Acetic acid.

Fig. 42. Three nuclei and two germ-cells found in a secondary Nematogen, which contained nine free nuclei. Acetic acid.

Fig. 43. Four nuclei and three germ-cells, found in a secondary Nematogen, which contained five nuclei. Acetic acid.

Plate 4.

Figs. 44—47 *Dicyemennea Eledones;* figs. 48—50 *Dicyemennea gracile;* figs. 51—59 *Dicyema truncatum.*

All the figs. magnified 465/1.

Fig. 44. Dorsal view of a Rhombogen. Parapolars (*pp*) short, alternating with the two proximate ectodermal cells. Osmic acid. Taken from *E. Aldrovandi.*

Fig. 45. The cephalic end of a living specimen (3.25 mm long). The head is .275 mm long and .225 mm wide. The parapolar collar broadest at its junction with the calotte. From *E. moschata.*

Fig. 46. Front view of the calotte, showing four ventral and five dorsal cells. Acetic acid. From *E. moschata.*

Fig. 47. Unliberated embryo (.2 mm long) from *E. moschata.* The 23 ectodermal cells seen in situ. Seen from one side. Cells of the body in alternating pairs. Osmic acid.

Dicyemennea gracile.

Fig. 48. From a young individual mounted in balsam (.6 mm long). Shows the central nucleus and eight germ-cells.

Fig. 49. Dorsal view of a typical calotte. Parapolars not thickened at their junction with the calotte. Acetic acid.

Fig. 50. Optical section of the calotte of a Rhombogen (3.2 mm long). The parapolars taper backward instead of forward as in fig. 44. Width of the calotte .06 mm; average width of the body .05 mm. Thickness of the ectoderm .005 — .007 mm.

Dicyema truncatum.

Fig. 51. Optical section of an embryo (.09 mm long) from *Rossia macrosoma.* The polar cells form a hollow hemispherical calotte of the same width as the body. Osmic acid.

Fig. 52. Caudal portion of an individual (.28 mm long) from *Sepia officinalis.* The two caudal cells are here much smaller than usual.

Fig. 53. A small example (.2 mm) from *S. officinalis.* There are only 12 cotodermal cells besides the calotte, in alternating pairs. The outlines of the polar cells were not clear. Osmic acid.

Fig. 54. A young individual (.2 mm) from *Rossia macrosoma,* with 14 ectodermal cells besides the polar cells. Osmic acid.

Fig. 55. Another young individual from *S. officinalis,* showing large pyriform caudal cells. From a specimen mounted in balsam.

Fig. 56. An unusual form from *S. officinalis.* Length .15 mm. Two large cells near the middle resemble the caudal cells. Mounted in balsam.

Fig. 57. An example (.25 mm long) from *S. officinalis.* Total number of ectodermal cells 22. Endoderm contained only 3 germ-cells. No central nucleus could be seen. The calotte has a lateral inclination. Acetic acid.

Fig. 58. Front view of the calotte, showing the alternate arrangement of the two sets of polar cells. From a Nematogen .5mm long. Osmic acid.

Fig. 59. Optical section of the head of a Rhombogen (.6 mm long) from *Rossia macrosoma*. Ectoderm charged with coarse shining granules of an elongated angular form. Calotte remarkably large for this species.

Plate 5.

Figures all from *Dicyema moschatum* where not otherwise stated.

Figs. 60—79 and 104—106 magnified 1060/1; the remaining figs., 465/1.

Figs. 60, 61. A mature vermific germ-cell and a two-cell stage from *Dicyema microcephalum*. The germ-cell .009 mm in diam., its nucleus .006 mm. Acetic acid.

Figs. 62, 63, 64. A small germ-cell (.0015 mm), a mature germ-cell (.0085 mm) with fusiform striated nucleus, and another mature cell showing the nuclear spindle in transverse section. Acetic acid.

Figs. 65, 66. The first two cleavage-stages. Acetic acid.

Figs. 67, 68. Two views of the three-cell stage. Acetic acid.

Figs. 69, 70. Two four-cell stages. Acetic acid.

Fig. 71. A somewhat later condition of the four-cell stage. Three cells now form a cap to the fourth cell (endoderm). Acetic acid.

Fig. 72. A five-cell stage — four ectodermal and one endodermal cell (*e*).

Fig. 73. A six-cell stage from *Dicyema microcephalum*. Acetic acid.

Fig. 74. Optical section of a Gastrula composed of 10 ectodermal cells and a central endodermal cell. The endodermal cell has not divided since the four-cell stage. Acetic acid.

Fig. 75. An optical section of a Gastrula composed of 13 cells. Acetic acid.

Figs. 76, 77. An optical section and a surface view of a Gastrula. Blastopore nearly closed. Acetic acid.

Fig. 78. A little more advanced stage. The cells on one side of the blastopore multiply more rapidly than on the opposite side. Acetic acid.

Fig. 79. A sub-spherical embryo from *Dicyemennea Eledones*. The endodermal cell contains a single primary germ-cell. Acetic acid.

Figs. 80, 81. An optical section and a surface view of the same pyriform embryo. The blastoporal side has an elongated pointed form. The broad end corresponds to the future cephalic end. Acetic acid.

Figs. 82, 83. Optical sections of still more advanced embryos. Osmic acid.

Figs. 84, 85. The nucleus of the endoderm in process of division. Fig. 85 magnified 1060/1. Acetic acid.

Figs. 86, 87. An optical section and a surface view of the embryo just after the first primary germ-cell has formed. Osmic acid.

Figs. 88, 89. Fig. 88 somewhat more advanced. It is about this time that the number of ectodermal cells becomes complete. Fig. 89 shows both of the primary germ-cells. Osmic acid.

Fig. 90. An embryo seen from one side. The polar and parapolar cells already distinguishable. Acetic acid.

Fig. 91. An embryo (.01 mm long) seen from the dorsal, or ventral side. Cilia already present. Osmic acid.

Fig. 92. A larger embryo with all the cells outlined. Acetic acid.

Fig. 93. Head of a young individual found free (.19 mm long). Osmic acid.

Fig. 94. The axial cell of a fully developed embryo (.15 mm long), showing two germ-cells that have arisen by division of the posterior primary germ-cell, and the anterior germ-cell still undivided. Osmic acid.

Fig. 95. From an embryo found free, in which the anterior primary germ-cell has been replaced by two cells. The fact that the primary cells do not divide simultaneously is to be connected with the fact that they do not originate at the same time. The first to arise is the first to divide. In figs. 96, 98, the germ-cells behind the central nucleus (*ne*) take the lead in dividing. This alternation of division between the two primary germ-cells (and their products) has been often observed. Osmic acid.

Fig. 96. The central portion of the axial cell of an embryo *Dicyemennea Eledones* still within the parent. Length .15 mm. One of the two cells behind the nucleus (*ne*) has divided. There is a considerable pause after each division, during which the division-products grow to the size of the original cell, similarly as in cleavage. Acetic acid.

Fig. 97. Central portion of a young *Dicyema macrocephalum* (.25 mm long). Here are seen two pairs of small germ-cells, and, in front of each pair, a single larger germ-cell. These pairs have evidently arisen by the division of germ-cells of the larger kind. Osmic acid.

Fig. 98. A somewhat less advanced condition of a still unliberated embryo. Acetic acid.

Fig. 99. Entire contents of the axial cell of a young *Dicyema truncatum* from *Rossia*. Length .3 mm. Acetic acid.

Fig. 100. The axial cell and contents of a young individual .3 mm long. Acetic acid.

Fig. 101. The axial cell and its contents at a later stage (*Dicyemennea Eledones*). Length .75 mm. Shows the arrangement of the germ-cells in a primary Nematogen. Acetic acid.

Fig. 102. A portion of a primary Nematogen, showing the central nucleus (*ne*) and the scattered germ-cells (*vg*). Acetic acid.

Fig. 103. Entire contents of the axial cell of another young Nematogen (.25 mm long). Osmic acid.

Fig. 104. A germ-cell in process of producing the paranucleus (*n'*). The fig. has not been correctly reproduced. The black dots separating *n'* from the central nuclear portion should have been more elongated. They appeared to represent remnants of spindle-fibres. Acetic acid.

Fig. 105. The paranucleus (*n'*) completely eliminated. Acetic acid.

Fig. 106. The paranucleus lies detached by the side of two cells which have arisen by division of the cell *m* in fig. 105. The membrane of the paranucleus is very thin. Acetic acid.

Fig. 107. A similar stage, in which the paranucleus has attained a distinctly double-contoured membrane. Acetic acid.

Fig. 108, 109. Two of the three young Infusorigens found in a Rhombogen 1 mm long. The two cells (*g*) of figs. 106, 107 multiply by division, and give rise to a group of cells consisting of a peripheral layer and a central cell. The paranucleus increases in size, and its membrane becomes very thick. Acetic acid.

Fig. 110. Entire contents of the axial cell of a young Rhombogen (1.15 mm long). The central nucleus (*ne*), the paranucleus (*n'*), and the Infusorigen.

In the central cell (germogen) of the Infusorigen are seen a few loose germ-cells and a large nucleus (n''). The Infusorigen represents a Gastrula like that of the vermiform embryo. Acetic acid.

Fig. 111. One of two Infusorigens from a Rhombogen 2 mm long. In front are seen two germ-cells in process of cleavage; behind, one germ-cell (*ig*), one 8-cell stage, one outlined embryo, and the parannucleus (n'). Acetic acid.

Fig. 112, 113. Two portions of the same axial cell (*Dicyemennea Eledones*, 2.4 mm), showing the final products of two Infusorigens. The germogen (*c*) consists of a granular body and a large nucleus (n''), and lies in the midst of small loose germ-cells, which are destined, after a period of multiplication by division, to become vermific germ-cells. Acetic acid.

Fig. 114. From a transitional 2.5 mm long, showing same elements.

Fig. 115. A similar portion from another individual 2.4 mm long. Acetic acid.

Fig. 116. Portion of the axial cell of an individual .7 mm long. An unusual form of the Infusorigen.

Fig. 117. A transitional condition similar to figs. 112—115. From an individual 1,45 mm long. Osmic. acid.

Fig. 118. A free germogen with several nuclei.